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ABSTRACTS

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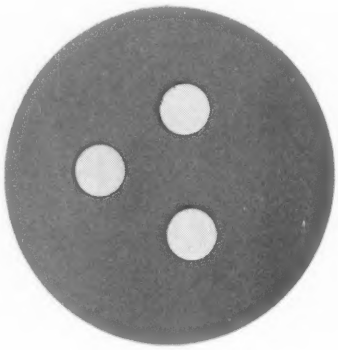

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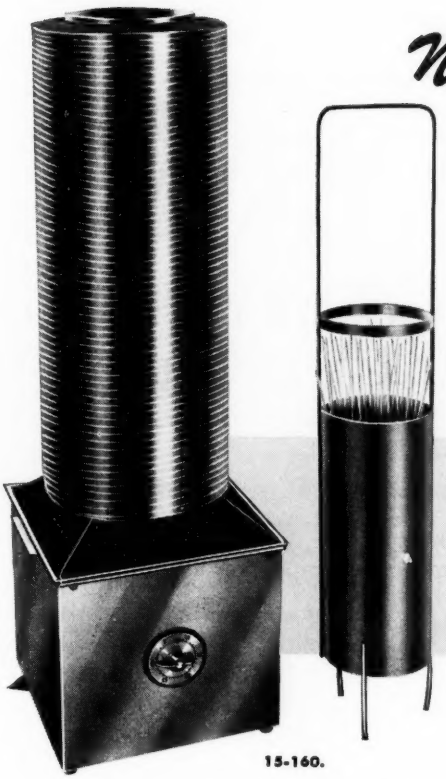

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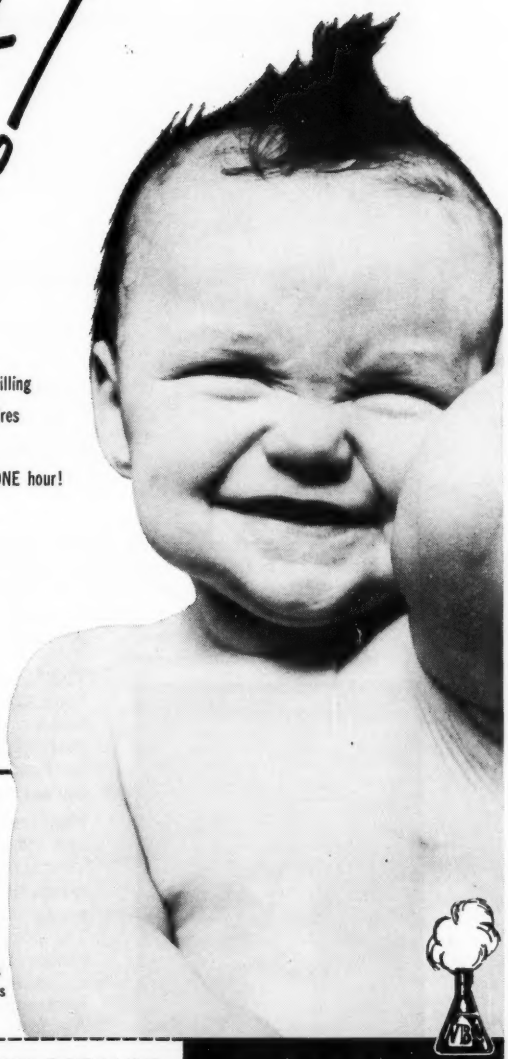

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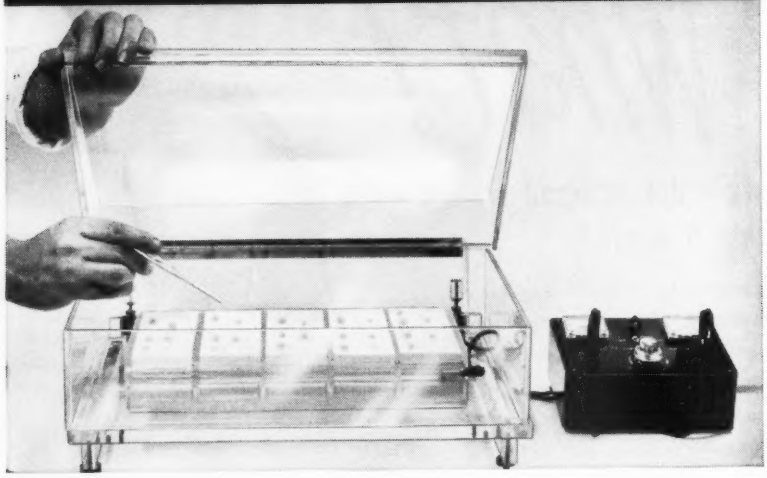

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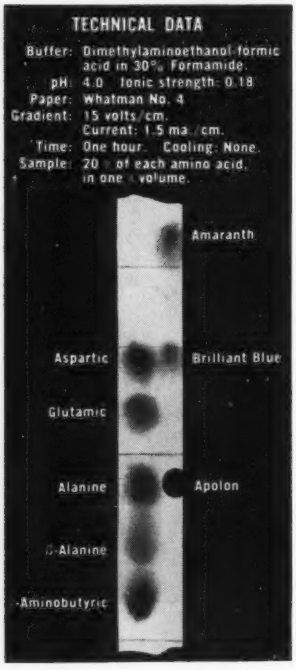

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*Rapid Paper Inophoresis Using Organic Buffers in Water-Formamide and Water-Urea. L. N. Werum, H. T. Gordon, W. Thornburg. *J. Chromatography* (in press).

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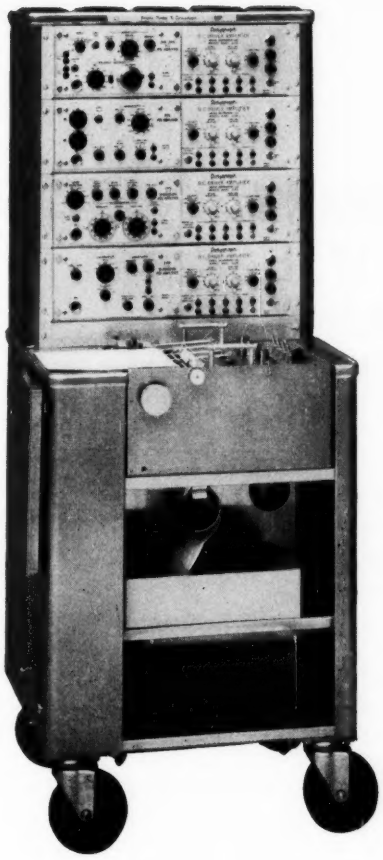

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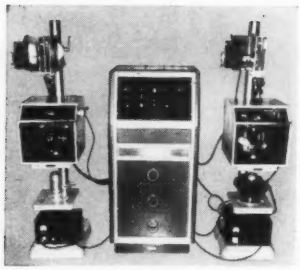

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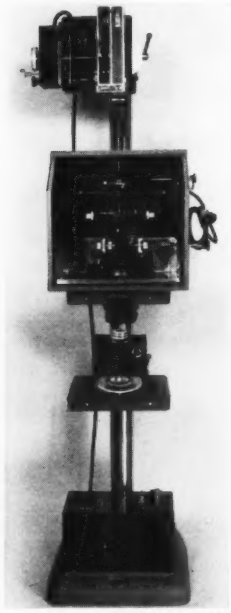

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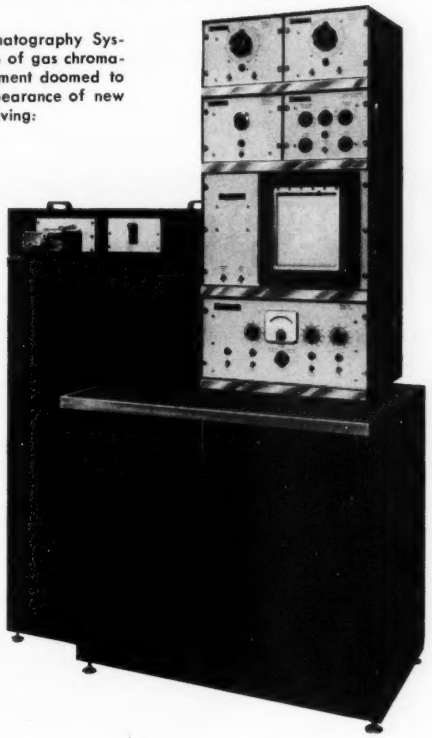

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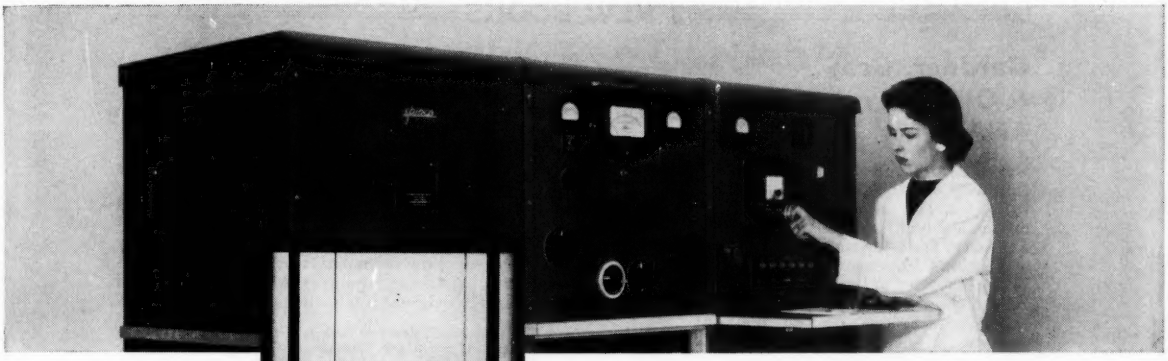
New Methods Extend the Usefulness of the Ultracentrifuge

Recent studies by research scientists have further increased the uses of the Analytical Ultracentrifuge for measuring molecular weights and purity of viruses, enzymes, proteins, polymers and a variety of organic and inorganic molecules. Here are four new developments as reported in the technical literature.



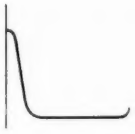
Interacting Systems

Kegeles and Rao at Clark University have measured the molecular weights of chemically reacting systems in the ultracentrifuge cell using the Archibald "approach-to-equilibrium" method. Studying the enzyme system alpha-chymotrypsin, they showed it to be present in the ultracentrifuge cell as an equilibrium mixture of monomers, dimers, and trimers. This is an extension of previous work which showed that the Archibald method applies to polydisperse non-ideal solutions, as well as to monodisperse ideal solutions.

Improved Accuracy

Trautman, at New York's Rockefeller Institute for Medical Research, showed that the accuracy of the Archibald method can be improved by more precisely locating the position of the meniscus on the ultracentrifuge photographic plate. He made a detailed study of the optical fine structure at the meniscus, and used a special optical aligning procedure with a mirror in the ultracentrifuge cell.

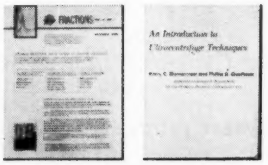
Simplified Measurements



At Stockholm's Nobel Medical Institute, Ehrenberg reports a simplified approach-to-equilibrium method which makes measurements from the schlieren curve easier. He runs the ultracentrifuge fast enough for a peak to begin forming at the meniscus so that the schlieren curve is parallel with the baseline and no extrapolation is necessary. His measurements of molecular weight and diffusion constants agree closely with those by other methods.

Rapid Equilibrium

Van Holde and Baldwin at the University of Wisconsin have used short liquid columns to achieve complete sedimentation equilibrium in a fraction of the time previously required. Using liquid columns of only 3 mm, they report equilibrium with sucrose in 3½ hours, and with a 1 mm column in only 30 minutes. In addition, the authors report that measurements during approach-to-equilibrium permit calculation of a diffusion coefficient.



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By ERNEST GARDNER, M.D., Professor and Chairman of Department of Anatomy, Wayne State University; DONALD J. GRAY, Ph.D., Professor of Anatomy, Stanford University; and RONAN O'RAHILLY, M.Sc., M.D., Associate Professor of Anatomy, Wayne State University. About 1024 pages, 7″ x 10″, with 632 illustrations.
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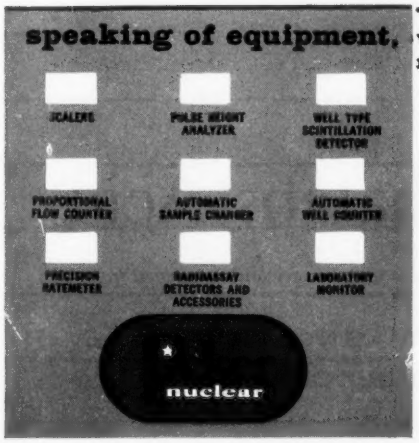

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ABSTRACTS

Babson, A. L., Read, P. A., and Phillips, G. E.: The importance of the substrate in assays of acid phosphatase in serum. Am. J. of Clin. Path. 32:1 83-87, July 1959.

A comparison is made between six generally used substrates for their relative specificity to prostatic and erythrocytic acid phosphatase. Results showed that a new substrate, alpha-naphthyl phosphate, was twice as specific as beta-glycerophosphate and 40-100 times as specific as all other substrates investigated. Acid phosphatase assays in serum should be specific for that enzyme arising from cancerous prostatic tissue. The substrate alpha-naphthyl phosphate* is specific for this enzyme.

Babson, A. L., and Read, P. A.: A new assay for prostatic acid phosphatase in serum. Am. J. Clin. Path. 32:1, 88-91, July 1959.

A new and specific method for the determination of prostatic acid phosphatase in serum is presented. The method is based on the use of a new substrate alpha-naphthyl phosphate which measures only prostatic acid phosphatase. The method is simple and rapid to perform with a minimum of manipulation. In this procedure the color developed represents prostatic enzyme activity alone. The reagents for this new method are available in convenient and stable form.*

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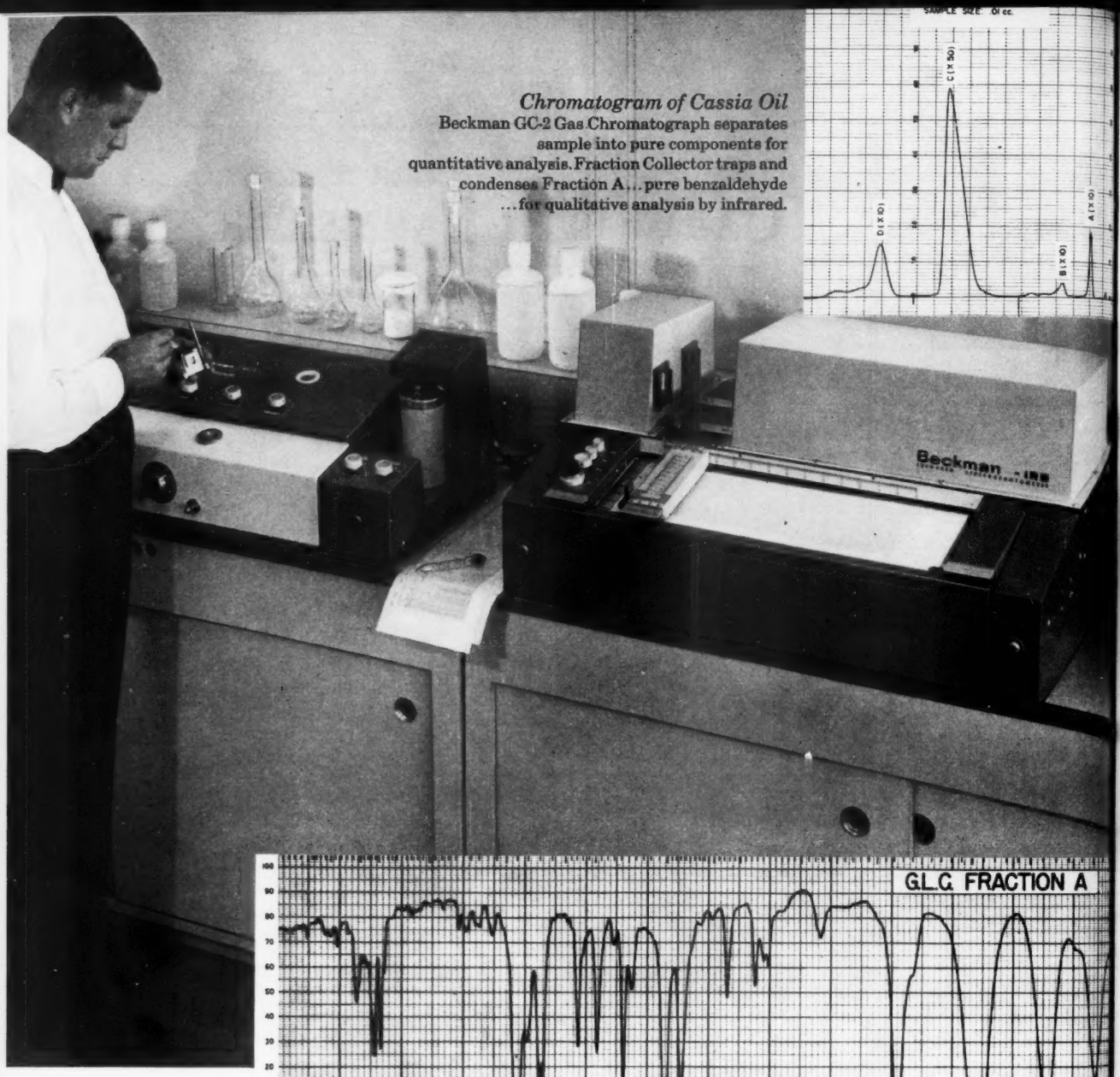

Chromatogram of Cassia Oil
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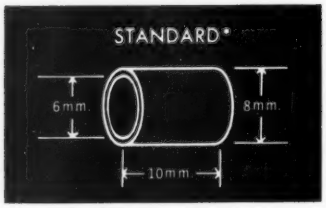

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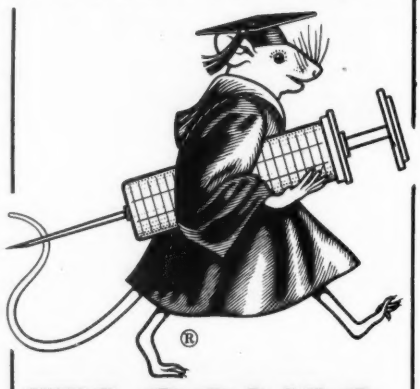

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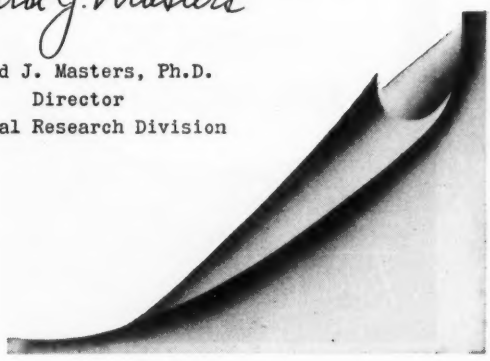

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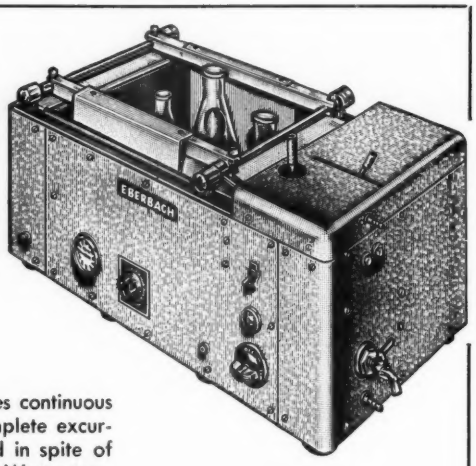

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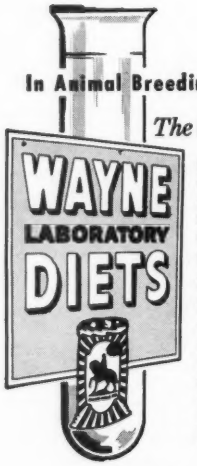

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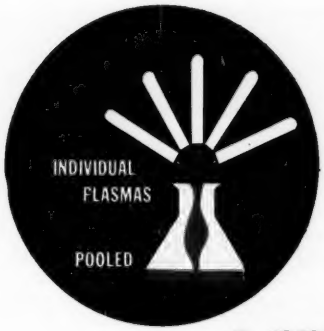
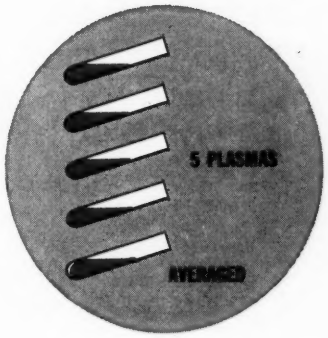


a prothrombin time control:
can be..., should be..., and why.



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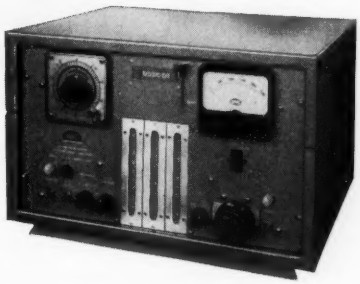

TYPES OF ACTIVITY 100% resolution of alphas in the presence of high-level beta activity. Betas accurately counted in the presence of alphas. No window absorption. Sample is analyzed directly inside detecting chamber. Mylar window detecting chamber optional at extra cost.

YIELD Full 2 pi geometry. Highest yield possible in flat sample counting — alphas 51%, betas 55% to 75%, gammas 1%.

PRECISION Detects every alpha and beta which escapes from the sample surface through a 2 pi angle.

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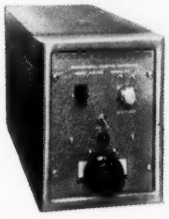
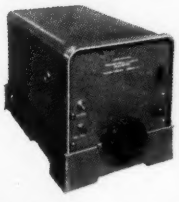
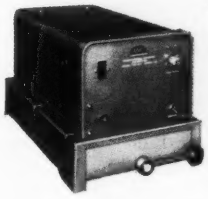


PCC-10A All-purpose counting chamber accepts disc samples up to 2¼" dia. x 7/16". Chambers available for lower background counting. 7½" x 10½" x 17" deep.



PCC-11A Chamber totally enclosed by heavy lead shield to reduce beta-gamma background counting. Other features are the same as for PCC-10A. 9½" x 9½" x 14½" deep.



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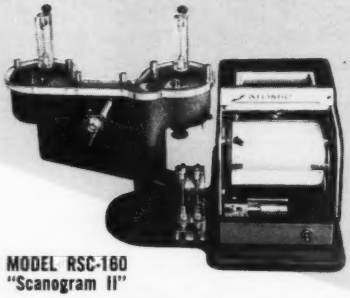

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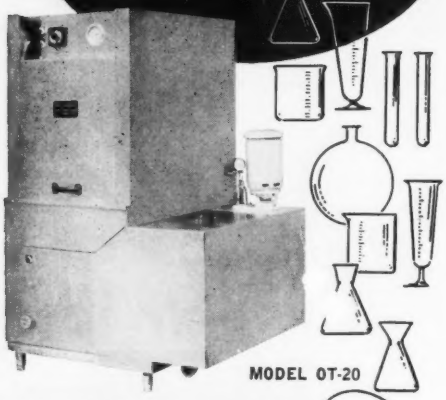

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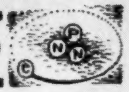
THYMIDINE 

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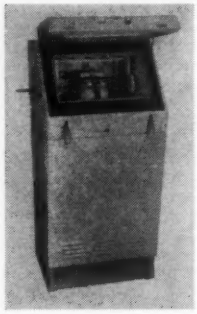

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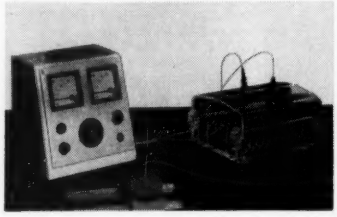

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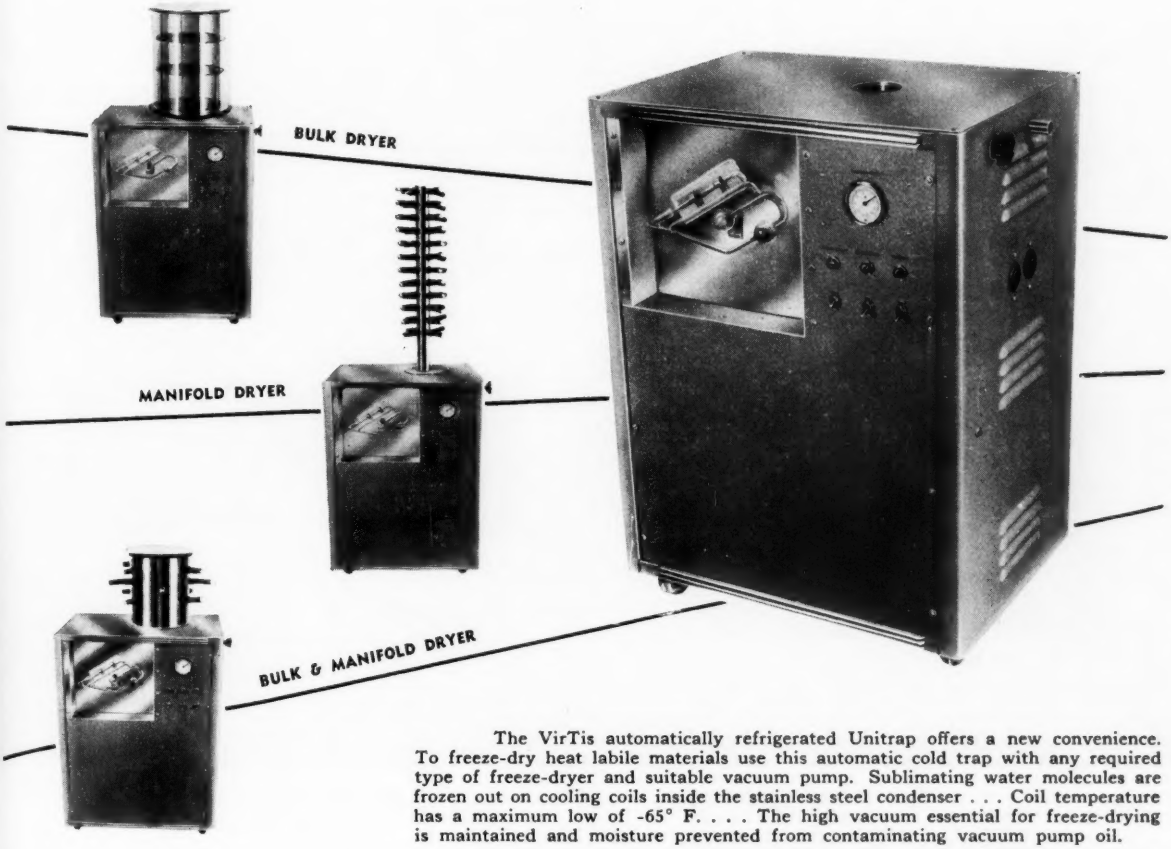

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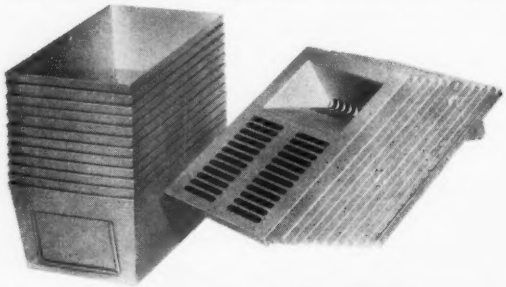

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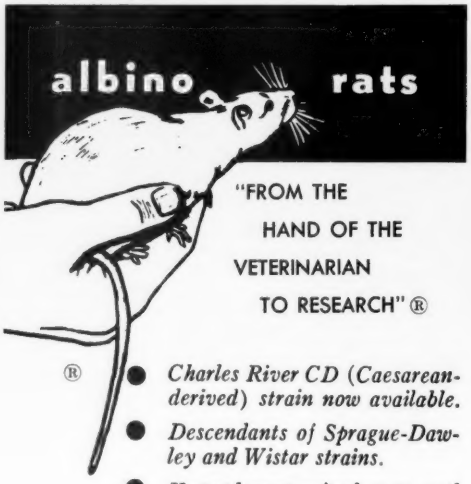

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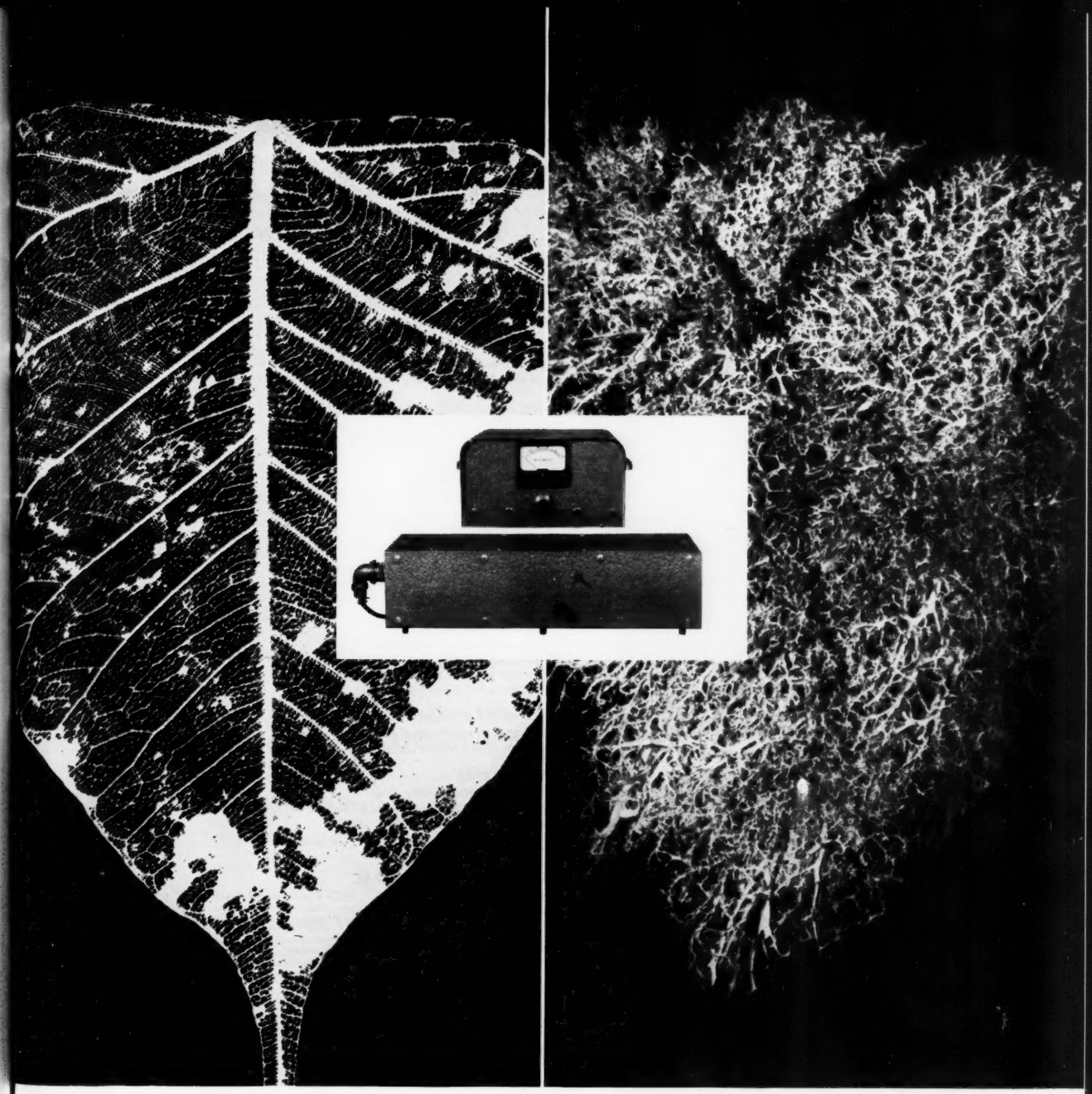

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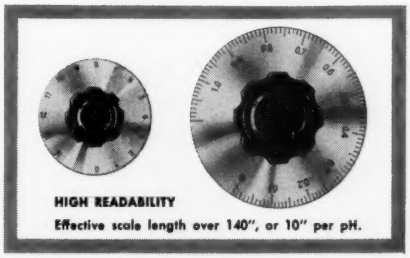

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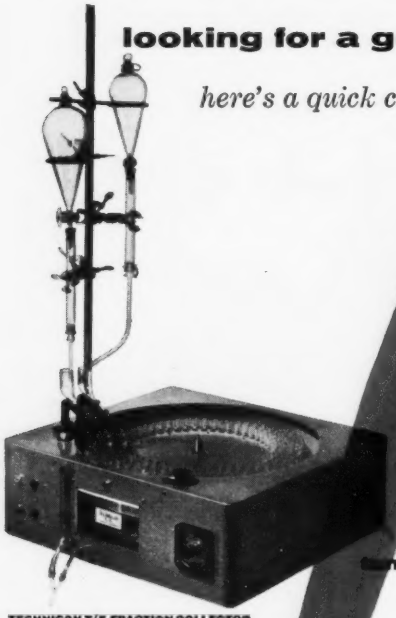

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FOREWORD

This year for the first time it has been possible to arrange the abstracts of papers for the Federation Meeting in a subject-classified order, integrated for all Societies. Previously they have been arranged alphabetically by first author by Society of origin; this made it difficult to retrieve particular information unless the user was aware of the first author of an abstract and the Society, even with the rudimentary subject index hitherto provided.

The ordering of the abstracts in this issue was done entirely by the Remington Rand UNIVAC Electronic Computer on the basis of the categories selected by authors from the list provided to them. Usually the first category selected by an author determined the location of his abstract, but sometimes for more precise placing the secondary choice was also involved. The subject index was entirely computer-generated from the authors' specifications and descriptive terms. The Society Secretaries and the Editor are well aware of the deficiencies of the classification system represented in the list of categories and absolve the computer of all responsibility for them.

Grateful acknowledgment is made to the National Science Foundation for their interest in and financial support of the experimental project (Grant NSF-G-9246); to Remington Rand UNIVAC, Philadelphia, for their generous contribution of personnel services and use of machine facilities; and to our printers, McGregor & Werner, Inc., Washington, for aid in working out the details of form and arrangement for publication.

Thanks are also expressed to the members of the library staff of Merck Sharp & Dohme Research Laboratories, West Point, Pa., for technical assistance in standardizing subject terminology; to Dr. Richard Orr, Institute for Advancement of Medical Communication, for aid in planning and guidance of the project; and especially to Mrs. Claire K. Schultz of the UNIVAC Applications Research Center, Remington Rand, for the detailed planning and execution of the project, under a rigid time limit, of harnessing the capabilities of a complex computer to the demands of a complex specific problem.

Finally the cooperation of authors in carefully following instructions for the submission of their abstracts and forms is gratefully acknowledged. Without this cooperation, the experiment would have been impossible.

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ERRATA

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001. AMINO ACIDS

1 *Physiology*

INVESTIGATIONS ON THE SEQUENCE OF AMINO ACID RESIDUES IN COLLAGEN. K. Hannig (intr. by P. D. Wall.) Max Planck Institut fur Eiweiss- und Lederforschung, Munich, Germany.

The highly differentiated intraperiod band fine structure of collagen seen in the electron microscope can be related to the molecular structure of this fibrous protein. Denatured procollagen was digested with a special purified, crystalline trypsin preparation. The complicated digestion mixture was fractionated by continuous paper electrophoresis and the homogenous fractions chromatographed on Dowex I. In this way a large number of basic, acidic and neutral peptides of high purity, having chain lengths ranging from 3 to 70 amino acid residues, were isolated. In contrast to previous investigations (in which the trypsin used for the digestion contained traces of chymotrypsin) the C-terminal groups were exclusively lysine and arginine, as might be expected. In practically all peptides the N-terminal group was found to be glycine and this amino acid made up almost one third of the fractions which favors an equal distribution of this amino acid within the chain. By enzymatic and chemical degradation methods the amino acid sequences were demonstrated to a large extent and showed that peptides rich in apolar regions (proline and hydroxyproline) are poor in polar regions (diamino or acidic amino acids) and vice versa. This result agrees with the electron microscope findings of K. Kuhn, U. Hofmann and W. Grassmann (Naturwiss., in press).

2 *Biochemistry*

BACTERIAL METABOLISM OF LYSINE. D. Rajagopal Rao, Gurmail Thind and Victor W. Rodwell (intr. by Harold Tarver.) Univ. of California School of Med., San Francisco.

In an attempt to clarify certain aspects of the metabolism of lysine by mammalian and bacterial systems, several strains of microorganisms capable of growth upon D,L-pipecolic acid as a sole source of carbon and nitrogen have been isolated from soil by the enrichment culture technique. Studies with one of these strains, Pseudomonas P-2, indicate that resting cell suspensions rapidly oxidize D- and L-pipecolate, D,L- and L-lysine, D- and L-glutamate, and D,L-α-aminoadipate. Growing cultures and resting cell suspensions utilizing D,L-pipecolate as substrate excrete α-aminoadipate and glutamate into the medium. These dicarboxylic acids have been separated by ion-exchange chromatography on Dowex-1-acetate and characterized by their infrared spectra, chemical behavior, and by their chromatographic and electrophoretic mobilities. In order to elucidate the mechanism of formation of α-aminoadipic acid, isotopic studies using lysine and pipecolic acid specifically labelled with C^{14} have been initiated.

3 *Biochemistry*

METABOLISM OF DL-PIPECOLIC ACID-2-C^{14} AND RELATED COMPOUNDS. Morton Rothstein and David M. Greenberg. Univ. of California School of Med., San Francisco.

DL-Pipecolic acid-2-C^{14} has been found to give rise to labeled α-aminoadipic acid in rat liver mitochondria. This finding establishes the position of pipecolic acid on the central pathway of lysine breakdown: lysine $\longrightarrow$ pipecolic acid $\longrightarrow$ α-aminoadipic acid $\longrightarrow$ glutaric acid $\longrightarrow$ acetate. A considerable amount (up to 10% of the starting activity based on L-pipecolic acid-2-C^{14}) of an unknown amino acid is obtained after chromatography of the incubation mixture on Dowex-50. The compound was first thought to be δ-amino-valeric acid, but has now been distinguished from the latter. Both α-aminoadipic acid-6-C^{14} and glutaric acid-1,5-C^{14} are readily oxidized to CO_2 by rat liver mitochondria in the presence of ATP and either Mg^{++} or versene. DL-Lysine-2-C^{14} itself does not appear to be oxidized to CO_2 to any great extent either by rat liver mitochondria or whole liver homogenate. Preliminary results, based on paper chromatography, appear to show that β-hydroxyglutarate is an intermediate in glutaric acid breakdown.

4 *Biochemistry*

ELEVATION OF γ-AMINOBUTYRIC ACID (GABA) IN BRAIN WITH HYDROXYLAMINE (NH_2OH). Claude F. Baxter* and Eugene Roberts. Dept. Biochem., Med. Res. Inst., City of Hope Med. Center, Duarte, Calif.

Administration of NH_2OH, a potent inhibitor of GABA-α-ketoglutaric acid transminase (GABA-T)(J. Biol. Chem. 233, 1135, 1958), to cats and monkeys (9.8 mg/kg I.V.) and to rats (49 mg/kg I.P.) increased GABA levels from 50 - 150% in several areas of brain. In rate this elevation was maximal at 1-1/2 hours and persisted for at least 24 hours. GABA-T activity, which was decreased at least 50% in rat cerebral cortex after NH_2OH (36 mg/kg; 90 minutes), was not restored in vivo by pyridoxine injection or in vitro by excess pyridoxal phosphate (PyP). GABA levels are regulated chiefly by glutamic decarboxylase (GAD) and the GABA-T systems. In the NH_2OH treated animals GAD activity was normal when assayed in presence of excess PyP. Elevated GABA levels were accompanied by significant increases in seizure thresholds in cats and monkeys. Pretreatment of rats with NH_2OH protected against convulsant doses of pentylenetetrazol for a period which coincided with the time during which elevated levels of GABA were found in cerebral cortex. Above results are not attributable to methemoglobinemia. The findings support the suggestion that GABA may play a role in regulation of neuronal excitability.

5 *Pharmacology*

POLYAMINE-GLUTATHIONE CONJUGATES IN E. COLI. Donald T. Dubin* and Sanford M. Rosenthal, National Institutes of Health, Bethesda, Md.

E. coli contains high levels of the triamine, spermidine (SPD) (H. Tabor, S. M. R., and C. W. Tabor, J. B. C. 233:907, 1958), and of glutathione (R. B. Roberts and E. T. Bolton, Science 115:479, 1952). In the present work a compound has been discovered which appears to be a conjugate between SPD and glutathione. The compound was extracted from washed cells with trichloroacetic acid, and was detected as a 2,4-dinitrofluorobenzene-reacting peak on Dowex-50 chromatography. It did not exchange with C^{14}-SPD in vitro, but contained radioactivity when isolated from cells grown in the presence of C^{14}-SPD or S^{35}-glutathione; it reacted with nitroprusside after treatment with cyanide; it traveled as a single ninhydrin-positive spot on paper chromatography; and after acid hydrolysis this spot was replaced by SPD and 3 other spots which traveled with glutamic and cysteic acids and glycine in 4 solvents. The conjugate contained ca. 20% of the SPD and over 50% of the acid-soluble S of the cells. Further studies on its properties will be described. An analagous compound containing the tetramine, spermine, was recovered from cells grown in the presence of this amine.

6 *Pharmacology*

ACTIVATION AND INHIBITION OF TRYPSIN BY QUATERNARY AMMONIUM COMPOUNDS. Brian C. W. Hummel (intr. by F. C. Lu). Food and Drug Labs., Dept. of Natl. Health and Welfare, Ottawa, Ont.

Certain quaternary ammonium compounds affect the activity of trypsin and thrombin (Nature 168:565, 1951). The effect of various such compounds on the rate of hydrolysis of p-toluenesulfonyl-L-arginine methyl ester (1mM, pH 8.1) was examined. Activation at all concentrations tested was observed with tetraethylammonium Cl, choline Cl, acetylcholine Br, succinyl choline Cl, penta- hexa- and decamethonium salts. The latter activated 87% at 30mM. Dodecylacetamidodimethylbenzylammonium Cl at 2mM gave 65% activation but at 14mM it caused 50% inhibition; a similar effect was observed with cetyltrimethylammonium Br, cetyl pyridinium Cl and tetra-n-butylammonium I. Inhibition occurred at all concentrations tested with ethylene bis (trimethylammonium) Cl, gallamine triethiodide (50% inhibition at 0.14mM) and domiphen Br. Protection of trypsin from denaturation at 40° by some of these compounds was observed but was less than that given by calcium. It is suggested that binding of certain types of quaternary ammonium compounds may shift the equilibrium between catalytically active and inactive trypsin towards the former.

XUM

7 *Pharmacology*

ACETYLATION OF POLYAMINES BY STAPHYLOCOCCUS. S. M. Rosenthal and D. T. Dubin*. Natl. Insts. of Health, Bethesda, Md.

Staphylococcus aureus (A.T.C.C. No. 152) grown in synthetic medium (modified from Fildes et al., Brit. J. Exp. Path. 1936, 17, 481) contains only small amounts of polyamines. When grown in the presence of C^{14}-spermine (0.07 mM) at 37°, 16 to 50% was concentrated in the cells, as free and conjugated amine. With C^{14}-spermidine less than 5% was present in the cells after 42 hours, nearly all as free amine. In the medium less than 5% of these amines was conjugated. When grown in the presence of C^{14}-putrescine (0.03 - 0.7 mM) essentially no label was present in the cells. In the medium however, high concentrations of mono-acetyl putrescine accumulated (50 to 75% in 20 hours and 95% in 40 hours). This acetylation was completely abolished when the cells were grown in neopeptone meat infusion broth. C^{14}-putrescine, spermine and spermidine were prepared synthetically (Tabor et al., J. Biol. Chem. 1958, 233, 907). Identification was accomplished by columnar and paper chromatography, and with acetyl putrescine, by recrystallization to constant activity and by stoichiometric amounts of acetate after hydrolysis. No significant oxidation of these amines was demonstrated.

8 *Nutrition*

CHEMICAL AND HISTOLOGICAL EFFECTS OF LYSINE DEFICIENCY ON THE FEMURS OF NEW HAMPSHIRE CHICKS. J. Edgar Braham*, Carlos Tejada* and Ricardo Bressani. Institute of Nutrition of Central America and Panama (INCAP), Guatemala, C. A.

One day old New Hampshire chicks were fed a sesame oil meal lysine-deficient ration alone or supplemented with graded levels of lysine. Four or five chicks were killed weekly and the femurs removed, cleaned of adhering tissue and immediately weighed. One was used for histological studies and the other for determination of moisture, ash, fat and protein content. As the lysine content of the ration decreased, a corresponding increase was found in bone fat and a decrease in moisture content. Neither the ash content nor the protein content of the organic residue changed significantly. Calcium and phosphorus in the ash remained constant under the different treatments and the Ca/P ratio did not change appreciably. Haemoglobin and hematocrit values showed a tendency to be inversely correlated with the lysine level of the ration. In experiments in which livers were removed and fat content determined, the lysine level of the ration did not affect the amount of fat deposited. The bone architecture was well preserved although the compact bone of the diaphysis and the epiphyseal cartilage was thinner in the lysine deficient animals. The red bone marrow was also reduced in volume.

002. AMINO ACIDS–Biosynthesis

1 *Biochemistry*

ACETYLORNITHINE δ-TRANSAMINASE: REPRESSIBILITY AND OTHER PROPERTIES. Alberta M. Albrecht* and Henry J. Vogel. Institute of Microbiology, Rutgers, The State University, New Brunswick, N.J.

In studies of ornithine and arginine synthesis, an enzymatic transamination of N-acetylglutamic γ-semialdehyde to N^{α}-acetylornithine has been noted (Vogel, Proc. Natl. Acad. Sci. 39:578, 1953). The enzyme involved, acetylornithine δ-transaminase, has now been extracted from Escherichia coli (strain W, grown on a glucose-salts medium) and partially purified. The (reversible) reaction can be studied in 0.1 M phosphate (pH 8.0), with N^{α}-acetyl-L-ornithine and α-ketoglutarate as substrates, for which the enzyme appears to be highly specific; pyridoxal phosphate is required as a cofactor. The enzyme activity is followed by assay of either the glutamate or the N-acetylglutamic γ-semialdehyde produced. Repression of acetylornithine δ-transaminase formation has been demonstrated upon cultivation of strain W in the presence of L-arginine. Acetylornithine δ-transaminase activity has also been found in extracts of E. coli, strain B; however, in this strain, no appreciable repression of the transaminase by arginine could be observed. (Aided by the Office of Naval Research, the U.S. Public Health Service, and the Damon Runyon Fund.)

2 *Biochemistry*

SUBSTRATES FOR YEAST SULFATE REDUCTASE AND STIMULATION BY A HEAT-STABLE FACTOR. Lloyd G. Wilson*, Tadashi Asahi* and Robert S. Bandurski. Dept. of Botany, Michigan State Univ., East Lansing.

Previous studies from this laboratory (J. Am. Chem. Soc. 80:5576, 1958) demonstrated an in vitro reduction of sulfate to sulfite using glucose-6-P, TPN and glucose-6-P dehydrogenase as the hydrogen donor system. An absolute requirement for ATP suggested that sulfate was activated prior to reduction and, in fact, 3'-phosphoadenosine-5'-phosphosulfate (PAPS) was found to substitute for ATP plus sulfate. Following partial purification of the reductase, PAPS and adenosine-5'-phosphosulfate (APS) both yield sulfite although APS does so at a slower rate than PAPS. The production of sulfite using PAPS, APS, or ATP plus sulfate as substrates is stimulated by the addition of a heat-stable, non-dialyzable factor from yeast. (Report of work supported, in part, by the National Science Foundation.)

3 *Biochemistry*

THE ROLE OF CARBONATE IN CITRULLINE BIOSYNTHESIS. M.E. Jones and L. Spector*. Brandeis Univ., Waltham, Mass.; Huntington Labs., Mass. Gen. Hosp., Boston, Mass.

The synthesis of citrulline with frog liver extracts proceeds by two reactions. In experiments with O^{18}-water reported by others (Metzenberg and Cohen; and by Reichard) and confirmed by us, one atom of O^{18} from water was incorporated into each of the two phosphate (P) molecules from equations 1 and 2 below. This incorporation could not result

1) $2ATP + NH_4HCO_3 \xrightarrow{\text{acetyl glutamate}} NH_2CO_2PO_3 + 2ADP + P$

2) $\text{carbamyl phosphate} + \text{ornithine} \longrightarrow \text{citrulline} + P$

from the hydrolysis of energy rich intermediates that are required for citrulline synthesis. It therefore seemed that the O^{18} from water must be in rapid chemical equilibrium with some substrate of the reaction, e.g., bicarbonate. We have found that the incorporation of O^{18} into P is almost completely inhibited by conditions which inhibit the equilibration of O^{18}-water with bicarbonate. Under these conditions, the O^{18} of labeled bicarbonate appeared in P. Once again both molecules of P were labeled with one atom of O^{18}. In view of the non-participation of the oxygen atoms of acetyl glutamate in the reaction, it is clear from these experiments that carbonate oxygen appears in P either through direct covalent bonding between carbonate and P or via intermediation of an enzyme oxygen.

4 *Biochemistry*

BIOSYNTHESIS OF C_4 ACIDS IN PSEUDOMONAS FLUORESCENS KB1. Chih H. Wang and George Ikeda.* Departments of Chemistry and Science Research Institute, Oregon State College, Corvallis, Oregon.

The relative contribution of CO_2 fixation (the $C_1 + C_3$ type) and the malate synthetase reaction to the biosynthesis of C_4 acids in Ps. fluorescens KB1 utilizing glucose as sole carbon source has been studied. Radiorespirometric studies with glucose-1, -2, -3, -3(4), or -6-C as substrate revealed that 85% of the administered glucose was catabolized via the Entner-Doudoroff pathway with the remaining 15% routed through a pentose involving pathway. Findings in the incorporation experiments with glucose-1, -2, -6-C^{14} or $C^{14}O_2$ as the respective carbon source indicated that the fixation of CO_2 onto pyruvate, as derived from glucose, is the predominate pathway in the biosynthesis of C_4 acids in this organism. Thus, C-1, C-2 (or C-6) and CO_2 were found to be incorporated heavily into the α-COH, the amino and methyl carbon atoms and the β-COH of aspartic acid respectively. The findings differ from that obtained in experiments using acetate as sole carbon source (Kornberg and Madson, Biochem. J. 68, 549 (1958)) in which malate synthetase was found to be playing an important role. (Supported by Atomic Energy Commission.)

5 *Nutrition*

BIOSYNTHESIS OF SERINE IN THE CHICK. Robert L. Wixom, Suzanne W. Conner* and W. Marcus Riddell*, Dept. of Biochemistry, School of Medicine, Univ. of Ark., Little Rock, Ark.

Nutritional studies have shown an interrelationship of serine and glycine for chick growth [Wixom et al., J. Nutrition 64, 13 (1958)]. However the pathway(s) of de novo biosynthesis of serine and glycine in the chick has not been previously investigated, as in the rat. Glucose-1-C^{14} was injected into normal and folic acid-deficient chicks, this deficiency being used to minimize the serine-glycine interconversion. The rate of production of respiratory $C^{14}O_2$ was the same in both groups for 4 hours. At this time, the specific activity of certain tissue components (lipid, protein, alanine, glutamic and aspartic acid from both the internal organs and the carcass) of the 2 groups had the same order of magnitude. Threonine and valine had a very low s.a. The s.a. of glycine was the same in both groups, whereas the s.a. of serine of the normal group was higher than that of the deficient. The significance of the findings will be discussed with regard to biosynthetic pathways.

.003. AMINO ACIDS–Chemistry

1 *Biochemistry*

EVIDENCE FOR A NEW AROMATIC AMINO ACID. J. E. Gander (intr. by F. E. Deatherage) Dept. of Agricultural Biochemistry, Ohio State Univ., Columbus.

When L-tyrosine was fed to sorghum a new aromatic amino acid was found. The material had the following properties: It was adsorbed onto activated charcoal from extracts of etiolated sorghum; was removed from activated charcoal with pyridine-ethanol-H_2O (10:50:40); and when exchanged onto Dowex-1-formate was removed only after elution with 1 M ammonium formate. The material reacted with ninhydrin giving a purple spot; it reduced ammoniacal silver nitrate; it reacted with diazotized sulfanilic acid forming a yellow product; it reacted with 2,4-dinitrophenylhydrazine only after treatment with 2 N NaOH, but would not react with ninhydrin after alkali treatment. The spectra in 1 N HCl, H_2O and 2 N NaOH was not the same as any of the known aromatic amino acids. Treatment with 2 N NaOH irreversibly shifted the absorption maximum from 275 mμ to 365 mμ. The activation and fluorescence spectra were similar to p-hydroxyphenacyl chloride. The infrared spectrum showed peaks of maximum absorption at 3.35, 6.20, 6.80, 7.05, 7.25 and 12.95 microns. These data present evidence for a new aromatic amino acid.

2 *Biochemistry*

THE INTERACTION OF ATROPINE WITH AMINO ACIDS.[1] Stephen I. Oroszlan*[2] and Gertrude D. Maengwyn-Davies. Dept. of Pharmacology, Georgetown Univ. Medical and Dental Schools, Washington, D. C.

Atropine in the presence of amino acids shows a marked increment in U.V. absorption with a maximum at 295 mu. The amino acids affording this spectral change are in order of decreasing activity: histidine, methionine, lysine, arginine, hydroxyproline, serine, glycine, alanine, and proline. This increment in absorption is inhibited by cysteine. This inhibition decreases with increasing atropine concentration in a manner similar to competitive enzymatic inhibition. It is probable that the increase in U.V. absorption at 295 mu is not due to hydrogen bonding since the atropine absorption at 258 mu remains essentially unchanged. The observed phenomenon would seem to be analogous to the reaction of quaternary pyridine derivatives with imidazoles forming an addition complex (J. van Eys, J. biol. Chem. 233: 1203, 1958).

(1 Supported by Grant B-1828, National Institute of Neurological Diseases and Blindness, U.S.P.H.S.
2 Predoctoral Research Fellow, National Institute of Neurological Diseases and Blindness, U.S.P.H.S., No. BF-10,852.)

3 *Pharmacology*

THE SOLUBILITY OF L-THYROXINE (Na) IN ETHANOL-WATER MIXTURES AT 25°C. H.E. Evert (intr. by Joseph G. Benton) State Univ. of New York College of Medicine, Brooklyn, N.Y.

The method reported by Evert, H.E., Federation Proc., 18, 224, 1959, for the determination of a "soluble" thyroxine in the presence of phosphate buffer and of neutral salts in aqueous media has been extended to include a study of the solubility properties of L-thyroxine (Na) in ethanol-water mixtures at 25°C. The determination of the relative solubility of amino acids in two different solvents frequently shows a simple and regular relationship to structure as pointed out by Edsall and Wyman, Biophysical Chemistry, v. 1, pp. 308-22, Academic Press, Inc., New York, 1958. The present work has been patterned after the work of Cohn, et al, J.Am.Chem.Soc., 56, 2270, 1934 and of Winnek, P.S. and Schmidt, C.L.A., J.Gen.Physical., 19, 733 and 773, 1935-36. Compared with other amino acids certain distinguishing features were noted when log N_3/N_3 was plotted as a function of the mole fraction of ethanol, N_2. Both the hydrocarbon component and the ionic groups in the structure appear to influence the solubility properties. The dielectric characteristics of the solvent media alter the electrical properties of the molecule. Therefore, when neutral salt is added to the ethanol-water system, it is possible to evaluate the possible interaction between ions and the dipolar ions which in turn reflect the electrostatic nature of thyroxine.

004. AMINO ACIDS–Metabolism

1 *Physiology*

EFFECT OF INSULIN ON AMINO ACID TOLERANCE. T. Hernandez and R. A. Coulson. L. S. U. Sch. Med., New Orleans, La.

Amino acids (10 mM/K) were injected into control alligators and into those primed with 100 U/K of insulin. Plasma amino acid levels were determined at intervals until the injected amino acid disappeared from the blood. Results are expressed as % of the disappearance rate of l-serine.[1] Insulin increased the rate of utilization of all amino acids of both isomers except d-leucine and d and l-tryptophane. Young rats metabolised amino acids 40 times as fast as the alligator. The relative order of utilization, however, was similar in both species. Diabetic alligators had elevated plasma amino acid levels. Insulin decreased, whereas S.T.H. and hydrocortisone increased both plasma amino acids and glucose. (Supported by NIH Grant No. H-2062)

Amino Acid	l	l+ Ins.	d	d+ Ins.
Ser	100	422	-	-
Gly	86	165	-	-
Ala	83	190	12	25
Orn	79	114	19*	30
$AspNH_2$	67	100	21	79
Arg	60	109	12*	54
Lys	54	200	11*	18
$GluNH_2$	32	83	30*	53
Leu	27	42	12	12
Try	17	17	16	16
Pro	14	84	-	-
Citrul	13	54	16*	24
Thr	11	83	12	46
Asp	9	15	7	13
His	8	35	8	11
Ileu	6	17	5	7
Glu	6	15	8	19
Met	< 5	10	14	15
Val	< 5	7	< 5	10

1 See Table *dl

2 *Physiology*

THE METABOLISM OF D,L-TRYPTOPHAN-β-C^{14} IN MAN AND IN RATS. Iris M. Dibner*, Aaron Feldstein*, Harry Freeman* and Hudson Hoagland. Worcester Found. Exper. Biol. and Medfield State Hospital.

D,L-Tryptophan-β-C^{14}, 5 μc, gave a 43.3±8.5% recovery in urine after i.v. injection to 4 schizophrenics and 31.7± 12.8% after oral intake by 5 schizophrenic patients. Radioautographs of two-dimensional paper chromatograms of neat urine showed only tryptophan; acid extracts showed indoleacetic acid, acetyltryptophan, indolelactic acid and 2 unidentified indoles. In isopropanol-ammonia, Unknown 1 was faster than indoleacetic acid by 0.10 units and Unknown 2 faster by 0.15 units: in butanol acetic acid the Rf's were similar.

D,L-Tryptophan-β-C^{14}, 15-25 uc, injected i.p. in 8 rats gave a recovery of 4.4±0.8% in urine. Forty (40) metabolites were characterized by Rf's on two-dimensional radioautographs. Indoleacetic acid, indoleacetyl glycine, indolecarboxylic acid, acetyltryptophan, kynurenic acid, and xanthurenic acid were identified. (Supported by grants from the Ford Foundation and USPH grant MY2967 and a USPH Research Fellowship BF7362-C2).

XUM

3 *Physiology*

STUDIES ON THE NATURE OF THE "ADAPTIVE" INCREASE IN TYROSINE α-KETOGLUTARATE TRANSAMINASE ACTIVITY OF RAT LIVER. Francis T. Kenney (intr. by A. Hollaender.) Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee.

Parenteral administration of hydrocortisone or of large doses of tyrosine to the rat results in a rapid 5- to 10-fold increase in the activity of hepatic tyrosine α-ketoglutarate transaminase (E. C. C. Lin and W. E. Knox, Biochim. Biophys. Acta 26:86, 1957). When homogenates of liver from such "adapted" rats are incubated with ATP and an ATP-generating system, a small but consistent increase in the activity of this enzyme can be observed in the early phases of incubation. Transaminase activity in homogenates of liver from nonadapted rats does not increase under similar experimental conditions. In an attempt to determine whether this in vitro increase, as well as the much larger in vivo increase in activity reflects a de novo synthesis of enzyme, specific antisera were prepared. The transaminase was purified 500- to 600-fold from rat liver by extensions of previously reported methods. Antisera were prepared in rabbits by administering small quantities of enzyme in Freund's adjuvant; the enzyme is quantitatively neutralized and precipitated by these antisera. Preliminary experiments indicate that the in vivo increase in transaminase activity following adaptation is not associated with a corresponding increase in antigenically reactive protein.

4 *Physiology*

INVESTIGATIONS OF A NEW FAMILIAL SYNDROME CHARACTERIZED BY AMINOACIDURIA AND DWARFISM. L.E. Rosenberg*, D. M. Watkin and P. S. Mueller*. Nat'l. Insts. of Health, Bethesda, Md.

A familial syndrome first described by Rowley, et al., characterized by aminosciduria, dwarfism & weakness has been investigated by detailed metabolic studies in a 12-yr-old boy, the propositus, 2 of whose siblings, also with growth retardation died at ages 7 & 8 respectively. Two brothers, aged 20 & 6, exhibit aminoaciduria but no other stigmata. The mother & half-sister display no signs or aminoaciduria. Ht & wt of the propositus are in the 3rd percentile for his age. He has no cataracts, hepatomegaly, splenomegaly or rickets, normal liver & renal functions & normal over-all carbohydrate, phosphate & calcium metabolism. He and his brothers excrete daily 5 to 20 times normal quantities serine, threonine, asparagine & histidine as well as less dramatic excesses of tyrosine, glycine, alanine, phenylalanine, ethanolamine and 1-methyl histidine. On a weighed diet containing daily 4 g protein & 100 cal/kg bbdy wt, the propositus gains about 50 g daily. Supplementation of this diet by 1 g threonine & 0.5 g lysine daily resulted in slight changes in wt gain and in N and calorie balances but marked increases in uric acid excretion. Renal clearance and protein tolerance studies suggest that the syndrome in the propositus may reflect his inability to incorporate into protein and/or oxidize amino acids.

5 *Biochemistry*

EFFECT OF SELENIUM ON METHIONINE FORMATION IN VIVO AND IN VITRO. Irene Rosenfeld. Div. Ag. Biochem., Univ. Wyo., Laramie, Wyo.

The daily intraperitoneal injection of 2 mg of selenium as sodium selenate/kg body weight produced chronic selenosis in Sprague-Dawley rats within four to five weeks. In vivo studies indicate that selenium interfered with protein synthesis in the liver. Total liver protein in non-seleniferous animals was 18.8%, while in chronic selenosis it decreased to 13.8%. In chronic selenosis the methionine content of liver was reduced over 30% when compared with normal liver. To further examine the mechanism responsible for methionine decrease in chronic selenosis, the effect of selenium on methionine synthesis in vitro was carried out according to the method of Dubnoff and Borsook, (J. Biol. Chem. 176, 789,(1948)). These results indicate that in rat liver homogenate sodium selenate or selenite inhibited transmethylation reaction of homocystine or homocysteine by betaine or choline. Selenite was a more active biological inhibitor than selenate. Larger yields of methionine was obtained when the methyl acceptor was homocysteine and methyl donor betaine, but the degree of inhibition by selenate or selenite was the same regardless of methyl donor or methyl acceptor used. Results in vitro studies suggest that the decrease of methionine in vivo was due to the action selenium on transmethylating system. (Aided by AEC, Contract No. AT(11-1) -630).

6 *Biochemistry*

COENZYME A AND HISTIDINE METABOLISM. Robert C. Baldridge and Ramon Burket*. Temple Univ. School of Med., Philadelphia, Pa.

One of the concepts of the enzymatic cleavage of urocanic acid involves addition of the elements of water to the carbon-carbon double bond of the imidazole ring followed by rearrangement of the H atoms. Such a reaction can be considered as involving a vinylogue of an unsaturated acid. As enzymatic hydration of unsaturated fatty acids occurs via the coenzyme A esters, it was of interest to determine the effects of depletion of tissue coenzyme A on levels of urocanase (and of histidase). Accordingly, rats were fed diets devoid of pantothenic acid and livers were analyzed for histidase, urocanase, rhodanese (as an index of protein synthesis) and coenzyme A. Similar data were obtained with livers of pair-fed control rats. Decreased levels of histidase and urocanase (significance, $P < 0.01$) but not rhodanese accompanied depletion of coenzyme A. Destruction of coenzyme A by aging of homogenates at 37° was accompanied by a loss of urocanase but not of histidase activity. It thus seems likely that the initial steps in the degradation of urocanic acid involve participation of coenzyme A, possibly as urocanyl CoA. Some non-CoA derivative of pantothenic acid may play a role in the straight deamination of histidine. (Supported by a grant from the National Institutes of Health, USPHS, Grant A-650 C5).

7 *Biochemistry*

SERINE-3-C^{14} AS PRECURSOR OF CHOLINE IN YEAST. George J. Alexander. N. Y. State Psychiatric Inst. and Columbia Univ., New York, N. Y.

The β-carbon atom of serine-3-C^{14} is a precursor of methyl groups of choline-C^{14} in yeast homogenates. Whether or not methionine (or its activated form) is an intermediate in this process is uncertain. The alternate pathway would require a transfer of the β-carbon atom of serine, as a folic acid complex, to aminoethanol or its derivatives. Our yeast homogenate required aminoethanol, folic acid, ATP, Mg^{++}, Vitamin B_{12} and homocysteine for activation. Addition of nonradioactive methionine, pyridoxal phosphate or Aminopterin was inhibitory. DPNH and CoA were without effect. S-adenosylmethionine decisively inhibited the reaction. Adenine and, to a lesser extent, hypoxanthine and 6-mercaptopurine, but not guanine, spared ATP. The requirement for homocysteine was quite specific. Other thiols tested were without effect. The homogenate has been subdivided. One fraction is capable of transmethylation but not of utilizing serine-3-C^{14} in choline synthesis. Another fraction does not synthesize choline-C^{14} although it consumes serine-3-C^{14}. The implications of these results will be discussed.

8 *Biochemistry*

ENZYMIC FORMATION OF CO_2 FROM THE CARBOXYL GROUP OF METHIONINE. Mendel Mazelis (intr. by S. Schwimmer.) Western Reg. Res. Lab., Albany, Calif.

Sulfur-containing amino acids have been implicated as the precursors of the volatile sulfur compounds responsible for the distinctive odor and flavor of vegetables in the Cruciferae, e.g. cabbage, and the Liliaceae, e.g. onion and garlic. An enzyme preparation which will convert the carboxyl group of methionine to CO_2 has been solubilized from the cytoplasmic particles of cabbage leaf homogenates. The system requires the addition of Mn++ and pyridoxal phosphate. When DL-methionine-2-C^{14} was the substrate, paper chromatography and radioautography of the reaction mixture revealed several radioactive products, however, β-methiopropylamine was not detected. When DL-methionine-$C^{14}OOH$ was incubated in the presence of γ-methio-α-ketobutyrate, no dilution of the labeled CO_2 produced was observed. Evidence has been obtained of the formation or participation of a peroxide in the reaction. The addition of catalase, peroxidase, or cytochrome C inhibits the reaction. Catalase is most effective as an inhibitor. Fe+++ has no effect. Addition of H_2O_2 or a peroxide-generating system to the reaction system is very inhibitory. A new type of pyridoxal phosphate-mediated amino acid decarboxylation is indicated.

9 *Biochemistry*

THE REACTION OF ALANINE AND HYDROXYASPARTATE WITH GLUTAMATE-ASPARTATE TRANSAMINASE. W. Terry Jenkins (intr. by C. E. Ballou) Dept. of Biochemistry, University of California, Berkeley.

Certain amino acids are incapable of reacting with the purified pig heart glutamic-aspartic transaminase. L-Alanine and β-hydroxyaspartate will, however, react very slowly with the pyridoxal form of the enzyme. The low activity with alanine is explained by the fact that alanine and pyruvate have very low affinities for their respective forms of the enzyme. The affinities of hydroxyaspartate and oxaloglycolate, however, are such that the enzyme-substrate complex (λ max 492 mμ) formed from either one, is effectively an enzyme-inhibitor type of complex. This unique absorption maximum suggests that the stability of the complex is due to increased resonance involving the hydroxyl group in the beta position. Resonance of this type is thought to explain certain elimination reactions catalyzed by pyridoxal phosphate. (Supported by grant RG-6315(A) from the N.I.H.)

10 *Biochemistry*

MAMMALIAN ORTHO-TYROSINE DECARBOXYLASE. Virginia E. Davis* and J. Awapara. The Rice Institute, Houston, Texas.

Several organs from different animal species (rat, rabbit, guinea pig, bovine, and pig) were found to decarboxylate o-tyrosine more actively than DOPA and 5-hydroxytryptophan. There is no agreement as to whether one enzyme or several enzymes are responsible for all these activities and the question of multiple specificity of DOPA decarboxylase has not been resolved. The activity of the enzymes was determined by measuring the amine formed after separation with an ion exchange resin. Some purification of the enzyme has been accomplished by fractionation with ammonium sulfate and adsorption with calcium phosphate gel. A nearly three-fold decrease in activity of DOPA decarboxylase relative to that of o-tyrosine decarboxylase was obtained. The evidence obtained so far indicates that DOPA and o-tyrosine are decarboxylated by different enzymes. (Supported by a grant from The Welch Foundation, Houston, Texas.)

11 *Biochemistry*

STUDIES ON A D-AMINO ACID OXIDASE DEFICIENT MUTANT OF NEUROSPORA. N. H. Horowitz, Eiji Ohnishi*, and Yasushi Watanabe*. California Institute of Technology, Pasadena.

The aim of this study is to investigate the role of the D-amino acid oxidase in metabolism. A single gene mutation that causes a deficiency of the enzyme was obtained by U.V. irradiation of a methionineless mutant which grows on D- as well as L-methionine. Selection was carried out for descendants unable to use the D-stereoisomer. A methionineless, D-oxidaseless double mutant was thus obtained. When combined with an arginineless gene, the D-oxidase mutation prevents utilization of the D-form in this case, too. The D-oxidase activity of the mutant is 3 to 5% of normal on nine different substrates. When extracted from its combination with methionineless, the D-oxidase mutant shows a block in sulfate reduction which is thought to be due to a coincident mutation of a gene close to the D-oxidase gene. Otherwise, growth of the mutant is normal. Attempts to detect accumulation of D-amino acids in extracts, medium, and cell wall of mutant cultures have so far given negative results. Inversion of exogenous D-amino acids is thus the only role established for the enzyme at this writing.

12 *Biochemistry*

ENZYMATIC SYNTHESIS OF γ-HYDROXYGLUTAMATE FROM L-HYLROXYPROLINE. Elijah Adams and Alfred Goldstone* Dept. of Pharmacol. St. Louis Univ. School of Med., St. Louis, Mo.

Two reactions in the metabolism of L-hydroxyproline by mammalian tissue have been outlined as (1)L-hydroxyproline → Δ^1-pyrroline-3-OH-5-COOH (HPC) (Taggart and Krakaur, J. Biol. Chem.177,671(1949)) and (2) HPC → γ-hydroxyglutamate (HGA) (Adams et al Biochim.Biophys.Acta,30,212(1958)). HPC has been prepared from L-hydroxyproline with beef-kidney mitochondria. Chemical characterization of purified HPC supports the structure originally postulated by Taggart and Krakaur. Purified HPC is enzymatically oxidized by a soluble dehydrogenase (liver) to yield HGA stoichiometrically. HGA, obtained as a crystalline, analytically-characterized enzymatic product, has the L-configuration at α- and γ-carbons, as expected from enzymatic derivation from L-hydroxyproline. Measured rotation of the product confirms the configuration assigned to this isomer, as obtained synthetically (Benoiton,et al, J.Am.Chem. Soc. 79,6192(1957)). The dehydrogenase, purified about 50-fold, is active with both DPN and TPN and oxidizes several Δ^1-pyrroline substrates: pyrroline itself, pyrroline-5-carboxylate and HPC. Competition studies suggest substrate-binding in the ring form; specificity studies suggest the name Δ^1-pyrroline dehydrogenase. The further metabolism of HGA is under investigation.

13 *Biochemistry*

THE MECHANISM OF ENZYMATIC FORMATION OF GLUTAMIC ACID (GA) FROM KYNURENIC ACID (KA). O. Hayaishi, S. Kuno,* M. Tashiro,* H. Taniuchi,* H. Yamada,* and K. Horibata.* Kyoto Univ., Japan. T. Sakan,* S. Seno,* and T. Tokuyama.* Osaka City Univ., Japan.

The formation of GA from KA by a partially purified enzyme from Pseudomonas was previously reported (O. Hayaishi et al, J. Am. Chem. Soc. 81:3483, 1959). KA-9-C^{14} yielded GA almost exclusively labeled in the α-carboxyl carbon while KA-2-C^{14} and -3-C^{14} produced GA labeled in carbons 2,3,4 and 5, indicating that the carbon skeleton of KA is not directly converted to GA. Upon purification of the enzyme, 7,8-dihydroxy-KA (I) was isolated from the reaction mixture and was identified by comparison with an authentic sample of I. I is converted enzymatically to an intensely yellow compound tentatively identified as β(2,6-dicarboxy-4-hydroxypyridyl-3)acrylic acid (II). II is then reduced enzymatically in the presence of TPNH to an as yet unidentified crystalline compound (III). M.p. 268-270°, $C_{10}H_{13}NO_6$ (C,49.93%, H,5.409%, N,5.312%), Emax=300 mu (water). Final structural proof of II and III is now in progress. (Supported by grants from U.S.P.H.S., Rockefeller Foundation and Jane Coffine Childs Memorial Fund).

14 *Biochemistry*

GLUTAMATE DEHYDROGENASES:VALIDITY OF SULFHYDRYL ESTIMATION;FACILITATION BY $CH_3Hg^{203}Br$. Paul J. Geiger*, Oscar K. Reiss, John Wein* and Leslie Hellerman. Johns Hopkins University, Baltimore.

Examination by applicable titration procedures has revealed (J. Biol. Chem., 233, 1468(1958)) that the chicken glutamate dehydrogenase possesses about 100 "subunits"(thiol equivalents) per mole of enzyme; and the beef enzyme, 40 such subunits with 80 equivalents titratable before denaturation and a total of 120 after guanidinium treatment. We have also reported an increased activity of the enzyme upon treatment with CH_3HgBr at pH 8.5. In order to examine the phenomena more fully we have synthesized $CH_3Hg^{203}Br$ and with this reagent have confirmed the above stoichiometric relationship for the thiol component. We have also confirmed the stoichiometry independently with rather specific oxidative titrations involving the use of o-iodosobenzoate, a porphyrexide, and an applicable iodimetric method. The validity of methods bearing on the essentiality of certain enzyme thiol fractions will be discussed. Concerning the second topic, facilitation of catalysis, we were able to show, with the aid of radioactive CH_3HgBr, that the major part of mercurial required to effect increased enzyme activity can subsequently be removed by dialysis with retention of the "facilitated" enzymatic rate. (Supported by National Cancer Institute Grant No. CY-3186.)

XUM

15 *Biochemistry*

ISOLATION AND ENZYMATIC REACTIONS OF AGARITINE, A NEW AMINO ACID DERIVATIVE FROM AGARICACEAE. Bruce Levenberg (intr. by Lila Miller.) Dept. of Biol. Chem., Univ. of Mich., Ann Arbor.

The press-juice of Agaricus bisporus (the mushroom of commerce) contains 0.03% agaritine, a new polyfunctional α-amino acid (percent composition: C,51.6; H,6.3; O,27.2; N,14.8. mol.wt.~300). Agaritine is a neutral, water-soluble compound that absorbs in the ultraviolet (λ_{max}=238 and 280 mμ; $\varepsilon = 10^4$ and 10^3, respectively). The presence of a reducing group in agaritine has been established from results of oxidation expts. with ferricyanide and periodate. The compound reacts with ninhydrin in the manner of a typical amino acid, liberating a mole of CO_2 at pH 4.7. Mild acid hydrolysis of agaritine results in the formation of a mole of L-glutamic acid. Agaritine interacts with glyoxylic acid in the presence of a soluble enzyme (purified 30-fold from extracts of A. bisporus) to form a new substance that yields a green chromophore with Ehrlich's DMAB reagent. Glyoxylate carbon is fixed into the product. The enzyme catalyzes analagous reactions between a variety of other carbonyl compounds and agaritine to form, in each case, a different ultraviolet-absorbing derivative. Agaritine and the enzymic component have thus far been detected only in basidiomycetes of the genus Agaricus. (This research is supported by a grant from the U.S. Public Health Service.)

16 *Biochemistry*

FORMATION OF N-FATTY ACYL AMINO ACIDS BY THE ACTION OF RAT LIVER ENZYME. Toshio Fukui* and Bernard Axelrod. Department of Biochemistry, Purdue University, Lafayette, Indiana.

Phenylalanine-C^{14} was previously shown to be incorporated into a lipid-like substance by isolated rat liver microsomes and particle-free cytoplasm (Axelrod, Haining and Fukui, Fed. Proc., 18, 184 (1959)). We have concentrated from rat liver acetone powder the enzyme which catalyzes this incorporation. This enzyme promotes the incorporation of phenylalanine into a lipophilic substance only in the presence of added lipid which is extracted from rat liver. The lipid can be replaced by lecithin or monoolein, but not by tricaproin or triolein. The product formed from phenylalanine-C^{14} and monoolein has been isolated by silicic acid column chromatography. It is stable to refluxing with 1 N ethanolic HCl or KOH for 1 hour, but it is hydrolyzed on heating with 6 N ethanolic HCl for 24 hours at 110° C, to yield radioactive phenylalanine. Carboxypeptidase also releases the phenylalanine from the lipophilic compound. Reduction with $LiAlH_4$ followed by acid hydrolysis gives a radioactive product with the same Rf as α-amino-β-phenylpropanol on paper chromatography. The specific activities on a weight basis of the isolated compound and the liberated phenylalanine suggest that the product is N-oleoyl phenylalanine. Similar compounds can be formed with other amino acids. Supported by NSF Grant G-5685.

17 *Biochemistry*

FURTHER PURIFICATION OF THE ENZYMES CONCERNED WITH THE BIOSYNTHESIS OF SPERMIDINE IN E. COLI. H. Tabor and C. W. Tabor. Natl. Insts. of Health, Bethesda, Md.

The following enzymatic steps are involved in spermidine biosynthesis in E. coli (H.Tabor,S.M.Rosenthal,and C.W.Tabor, J. Biol. Chem. 233, 907, 1958): I. Methionine + ATP $\xrightarrow{Mg^{++}}$ Adenosylmethionine (AMe) + P-P + P; II. AMe $\xrightarrow{Mg^{++}}$ Decarboxylated AMe + CO_2; III. Putrescine + Decarboxylated AMe → Spermidine + Methylthioadenosine. Enzyme I has been purified 1500x; this prepn. was free of pyrophosphatase. Isotopic studies demonstrated that the terminal P of ATP^{32} is converted to ortho-P; this confirmed the findings of Cantoni et al. with liver and yeast enzymes. No dissociation of the activity into partial reactions could be observed. Enzyme II has been purified 20x, and was used for the prepn. of decarboxylated AMe (80% yield). Enzyme III (propylamine transferase) has been purified 1000x. The purified prepn. was free of the enzymes splitting methylthioadenosine that are in crude prepns., and permitted the accumulation of methylthioadenosine. This was demonstrated in an experiment in which C^{14}methylthioadenosine and spermidine were formed from $C^{14}H_3$-decarboxylated AMe and putrescine. The methylthioadenosine was identified by chromatography (with carrier methylthioadenosine) on XE-64(H^+) and Dowex-50(H^+) columns, paper chromatography in two solvents, and constancy of specific activity upon recrystallization.

18 *Biochemistry*

METABOLISM OF C^{14}-CARBOXYL-LABELED-3-HYDROXYANTHRANILIC ACID AND PARA-AMINOBENZOIC ACID-1-C-14 IN NEOPLASTIC MICE. L.V.Hankes, M.Schmaeler* and R.D.Stoner,* Med.Res.Center, Brookhaven Natl.Lab.,Upton, L.I.

C^{14}-carboxyl-labeled-3-hydroxyanthranilic acid (C-14-3-OHAA) and para-aminobenzoic acid-1-C-14 (PAB-1-C-14) were synthesized. These compounds were injected by intraperitoneal route into (a) normal mice and mice with (b) spontaneous mammary tumor (c) ascites tumor and (d) transplantable brain tumor to study the effects of the tumors on metabolism of these compounds. C-14-3-OHAA was given in a dose of 0.0011 mM containing 0.024 c of activity per gram body weight. PAB-1-C-14 was injected in a dose of 0.0017 mM containing 0.024 c of activity per gram. Respired CO_2 collected for the first 12 hours showed that in (a) 44%, (b) 46%, (c) 40%, and (d) 41% of the injected C-14-3-OHAA activity appeared in expired air, while almost none of PAB-1-C-14 was metabolized to CO_2. The C^{14} activity level in the urines of mice given C-14-3-OHAA was (a) 30%, (b) 27%, (c) 22%, and (d) 20% of the injected dose. The C^{14} activity level in the urine of mice given PAB-1-C-14 was (a) 88%, (b) 97%, (c) 96%, and (d) 87% of the injected dose. Microbiological assays of the urines showed that approximately 15% of the labeled 3-OHAA appeared as quinolinic acid in (a), 6.5% in (b), 2.2% in (c) and 6.7% in (d).

19 *Biochemistry*

IMPAIRED CONVERSION OF PHENYLALANINE TO TYROSINE AFTER FOLIC-ANTAGONIST THERAPY IN WOMEN. Theodore L. Goodfriend* (intr. by F. Tietze.) Natl. Insts. of Health, Bethesda, Md.

Tetrahydrofolate and related tetrahydropteridines have been shown to participate in the enzymatic conversion of phenylalanine to tyrosine. (Kaufman, J. Biol. Chem. 234: 2677, 1959). Oral phenylalanine tolerance tests were performed in 12 women before, during, and after amethopterin therapy for choriocarcinoma. Serum phenylalanine was measured enzymatically, and tyrosine was measured using the nitrosonaphthol procedure. When 0.1 gm/kg of L-phenylalanine was fed following therapy, the peak serum values were 0.7-1.0 μM/ml, double the peak values before therapy, and the curves indicated a delay in disappearance. Plasma tyrosine levels rose to one half the levels seen before therapy. Fasting values of these amino acids were unchanged. Urinary phenylpyruvate excretion was negligible. These effects were evident within 12 hours after the first injection of folic antagonist. The degree of impaired phenylalanine tolerance did not correlate with the toxic or therapeutic effects of amethopterin in these patients. These results are comparable to those found in vitro and in the intact rat, (Kaufman and Levenberg, J. Biol. Chem. 234: 2683, 1959), and suggest that a pteridine cofactor is active in phenylalanine hydroxylation in the human.

20 *Biochemistry*

SEPARATION OF THREE TRANSAMINASES FROM DOG LIVER. Gerard A. Fleisher. Section of Biochemistry, Mayo Clinic and Mayo Foundation, Rochester, Minnesota.

By the use of cellulose ion exchangers or paper electrophoresis it was found that glutamic-oxalacetic transaminase activity in liver and heart is due to mixtures of two enzymes (Fleisher, Potter and Wakim, Proc. Soc. Exper. Biol. & Med., in press) referred to as GOT-I and GOT-II. Anionic GOT-I is the previously known enzyme; cationic GOT-II is a new type. They differ also in solubility and substrate affinities. Glutamic-pyruvic transaminase (GPT) seems to occur in one form only. A method has been developed for the simultaneous preparation of GOT-I, GOT-II and GPT from dog liver. It includes adsorption of GOT-I and GPT on DEAE-cellulose, leaving GOT-II in the filtrate, elution with 0.9% NaCl and ammonium sulfate fractionation at 0.55 and 0.85 saturation to separate GPT and GOT-I. Further purification steps including specific heat treatments for GOT-I and GPT, and ammonium sulfate fractionations for all three, lead to more than 100-fold concentration of each transaminase.

21 *Biochemistry*

INFLUENCE OF DIETARY AROMATIC AMINO ACIDS ON PHENYLALANINE HYDROXYLASE ACTIVITY. R.A. Freedland*, M.C. Krakowski*, and Harry A. Waisman. Sarah A. Workman Laboratory, Dept. of Pediatrics, Univ. of Wisconsin Med. School, Madison, Wis.

It had been reported (Nature 182, 871, 1958) that 5% DL phenylalanine or 5% L tyrosine added to commercial rat diet caused almost complete loss of phenylalanine hydroxylase activity in white rats after about 16 weeks. Using an improved method for this enzyme we have studied the influence of age, sex, and diet on enzyme acitivity. Our newer results have confirmed decreased activity after excess phenylalanine feeding, but only of the order of 50%. Excess tyrosine and/or tryptophan feeding consistently caused an increase in activity. Studies relating length of time on diet to activity indicated that the enzyme response is rapid, but does increase with time up to 10 weeks. A 16 hour fast completely abolished the phenylalanine effect until after the rats had been on the diet for 4 weeks. Various combinations of phenylalanine, tyrosine, and tryptophan in the diet were examined for their effect on phenylalanine hydroxylase activity. Fed and fasted results were obtained with these combinations at 4 and 16 weeks. Phenylalanine in the presence of either of the other two always produced a decrease in activity, whereas either or both tryptophan and tyrosine in the absence of added phenylalanine produced an increase in activity.

22 *Biochemistry*

S-METHYL METHIONINE: A MAJOR METHIONINE METABOLITE IN THE JACK BEAN. Ronald C. Greene (intr. by G. W. Schwert.) VA Hospital and Duke University Medical Center, Durham, N. C.

When intact jack bean seedlings are grown with C^{14} or S35-methionine for short periods of time, up to half of the acid-soluble radioactivity can be found in a single basic compound. This compound, located primarily in the roots, reaches maximal concentration during the first day and slowly disappears on subsequent days. The compound was isolated by chromatography on columns of Dowex 50 and Amberlite IR 120 using ammonium formate as the eluting agent, followed by paper electrophoresis in borate buffer. The isolated compound is indistinguishable from authentic S-methyl methionine chloride by paper chromatography in four systems and by paper electrophoresis. S-methyl methionine and the isolated compound give identical degradation products upon hydrolysis with HCl or NH_3 or reduction with sodium borohydride. Incubation of excised jack bean roots with labeled methionine, in a medium containing all the factors necessary for root growth, results in incorporation of the label into S-methyl methionine at a rate comparable to that observed in the intact plant. The incorporation is complete within twelve hours. The properties of this system and some of the metabolic reactions of S-methyl methionine in the jack bean are being investigated. (Supported by Grant No. RG-5509 from the U. S. Public Health Service.)

23 *Biochemistry*

SYNTHESIS AND METABOLISM OF S^{35}-(DICHLOROVINYL)-L-CYSTEINE. M. O. Schultze and R. F. Derr.* Dept. of Agricultural Biochemistry, Univ. of Minnesota, St. Paul.

S-(dichlorovinyl)-L-cysteine (DCVC) induces in very small amounts (3 mg/kg, I.V.) fatal aplastic anemia in calves (Blood 14, 1015 (1959)); most other species thus far tested are much less susceptible to its toxic effects. To study its localization in tissues and its metabolism, DCVC was labeled with S^{35}. H_2S^{35} was converted to potassium benzylmercaptide-S^{35}, the latter condensed with L-2-amino-3-chloropropionic acid to form S^{35}-benzyl-L-cysteine which, after treatment with sodium and trichloroethylene in liquid ammonia afforded DCVC-S^{35}, spec. activity 2×10^6 C.P.M./mg. After oral or I.P. administration to rats, radioactivity from DCVC-S^{35} was excreted during the first 24 hours in the urine in 3 main fractions, partly as DCVC, partly as sulfate and partly as a butanol-soluble derivative. After 3 or 21 days the highest radioactivity per gm. of tissue was in kidney, liver and heart, less in bone marrow. Aerobic incubation of DCVC-S^{35} with homogenates of rat liver in tris buffer caused rapid formation of sulfate and combination of radioactivity with ethanol-insoluble components of the tissue; with homogenates of rat kidney formation of sulfate was less rapid and extensive. (Supported in part by U. S. Atomic Energy Commission, Contract AT (11-1)-364).

24 *Biochemistry*

L-AMINO ACID DECARBOXYLASE ACTIVITY IN MAMMALIAN TISSUES AND ITS INHIBITION BY α-METHYL DOPA. S. Udenfriend, W.M. Lovenberg*, and H. Weissbach* Natl. Heart Inst., Bethesda, Md.

Tryptamine, phenylethylamine and ortho, meta and para tyramine have now been definitely identified as normal excretory products in man and experimental animals. Investigation of their biogenesis has led to the finding that decarboxylation of all the naturally occurring aromatic amino acids takes place in mammalian tissues. With partially purified guinea pig kidney preparations the following results were obtained (the figures in parenthesis indicate amine formation in terms of μg/mg protein/hr.): 5-hydroxytryptophan (1000), 3,4-dihydroxyphenylalanine (DOPA) (3000), tryptophan (40), phenylalanine (15), tyrosine (2), and histidine (1). Previous reports from this laboratory suggesting separate DOPA and 5-hydroxytryptophan decarboxylases must now be withdrawn. Existence of a single aromatic L amino acid decarboxylase is suggested by the persistence of all these activities with purification (25 fold), competitive inhibition among substrates and inhibition of all decarboxylations by α-methyl DOPA. The latter compound was found to be decarboxylated to the same extent as tryptophan. Other α-methyl amino acids were also decarboxylated, indicating that the hydrogen on the α carbon is not necessary for decarboxylation.

25 *Biochemistry*

TRACER STUDIES OF S-METHYLMETHIONINE-HOMOCYSTEINE TRANSMETHYLASE IN HIGHER PLANTS. James E. Turner (intr. by Robert W. Swick.) Argonne National Laboratory, Lemont, Ill.

The enzyme catalyzing the transfer of a methyl group from S-methylmethionine to homocysteine has been detected in mammalian liver and certain micro-organisms. Through the use of the assay method of Shapiro and Yphantis (Biochem.Biophys. Acta, 36: 241(1959)), this transmethylation has now been observed in cell-free homogenates of higher plants. S-Methyl-L-methionine (methyl-C^{14}) was used as the methyl donor, and the methionine formed was identified by paper chromatography. Homogenates prepared from the dry, mature seeds of Phaseolus vulgaris, Pisum sativum, and Zea mays showed transmethylase activity when homocysteine was added as a methyl acceptor. A preliminary survey of a variety of plant species indicates that the methylmethionine-homocysteine transmethylase system is not a rare enzyme but exists in the seeds of several higher plants. Cabbage (Brassica oleracea) contains S-methyl-L-methionine but no transmethylase activity was detected in the seeds or leaves with either homocysteine or cysteine as methyl acceptor. (Work was performed under the auspices of the U.S. Atomic Energy Commission.)

26 *Biochemistry*

ENZYMATIC N-METHYLATION OF CARNOSINE. I. Rosabelle McManus, Dept. of Biochem. and Nutr., Univ. of Pittsburgh, Pittsburgh, Pa.

In vivo studies (J.Biol.Chem., 225, 325, (1957)) showed that methionine-$C^{14}H_3$ is incorporated into rat muscle anserine. An enzyme preparation from chick pectoral muscle, which catalyzes the incorporation of C^{14} from S-adenosylmethionine-$C^{14}H_3$ (S-AMe) into the 1-methylhistidine moiety of anserine, was obtained by homogenization of tissue in 0.3 M sucrose followed by centrifugation at 7,000 g. The supernatant, when supplemented with ATP and L-carnosine in tris buffer, pH 7.4, also catalyzed the utilization of L-methionine-$C^{14}H_3$, indicating the presence of the methionine-activating enzyme. Identity of labeled product was established by paper and column chromatography with carrier before and after hydrolysis, and by the constant specific activity of 1-methylhistidine dipicrate. Utilization of the methyl of S-AMe was stimulated by increasing concentrations of L-carnosine although L-histidine, Mg^{++}, and GSH were ineffective. Transferase activity also occurred in chick liver and in rat, guinea pig, and cat muscle and liver. Kidney had little activity. The relation of this carnosine N-methyl transferase to imidazole N-methyl transferase (J. Biol. Chem., 234, 2948, (1959)) and to a similar enzyme described by Winnick et al (Nature, 183, 1466, (1959)) is under investigation.

XUM

27 **Biochemistry**

ENZYME INHIBITION FROM FEEDING L-TYROSINE TO SCORBUTIC GUINEA PIGS. Bert N. La Du and Vincent G. Zannoni*. Natl. Inst. of Arthritis and Metabolic Diseases, Bethesda, Md.

Scorbutic guinea pigs and man excrete tyrosyl compounds if fed extra phenylalanine or tyrosine. This is apparently due to an inhibition of p-hydroxyphenylpyruvic acid oxidase, one of the enzymes in tyrosine metabolism. The latter enzyme is inactivated by excess p-hydroxyphenylpyruvic acid and the inhibition can be prevented by vitamin C both in vitro and in vivo (J. Biol. Chem., 234:2925,1959; J. Biol. Chem., in press). Studies have been made on the effect of feeding tyrosine on tyrosine transaminase, p-hydroxyphenylpyruvic acid oxidase and homogentisic acid oxidase in liver and kidney. Scorbutic guinea pigs fed extra tyrosine had over 80% of their liver p-hydroxyphenylpyruvic acid oxidase inactivated within 2 hours, whereas animals receiving vitamin C had no reduction in oxidase activity. Plasma and urinary tyrosyl compounds were measured and correlated with these changes in enzyme activity. Tyrosine transaminase levels in scorbutic animals fed extra tyrosine were significantly higher than in normal controls. This increase favors the accumulation of p-hydroxyphenylpyruvic acid, and the accumulated acid leads to inactivation of the oxidase. These studies demonstrate that at the time of a vitamin deficiency a normal dietary amino acid may have a toxic effect on an enzyme system involved in its metabolism.

28 **Biochemistry**

THE TRYPTOPHANE METABOLISM OF CHICKENS. Y. Matsumura. (intr. by S. Lepkovsky.) Dept. of Poultry Husbandry, Univ. of Calif., Berkeley, Calif.

The hormonal control of the carbohydrate metabolism of the chicken is different from that of man and laboratory animals such as rats, cats and dogs. Several workers have found that the tryptophane and its metabolites show some relation to the metabolism of carbohydrates. In this study we investigated the tryptophane metabolism of depancreatized chickens and of intact chickens and rats. Chicken urine was collected through an artificial anus. Kynurenine, anthranilic acid and xanthurenic acid were determined in 24 hour urine samples in 3 groups (5 animals in each group) and presented in the table in micrograms per 100 grams weight of animal.

KIND OF ANIMAL	KYNURENINE	XANTHURENIC ACID	ANTHRANILIC ACID
Rats	773	1201	385
Depancreatized Chickens	1438	242	180
Intact Chickens	2615	343	271

It may be concluded that the chicken, compared to the rat, is limited in its ability to metabolize kynurenine, causing it to accumulate in its urine. This is reflected in a marked decrease in the excretion of xanthurenic and to some extent of anthranilic acid. Pancreatectomy of chickens seems to decrease the excretion of tryptophane metabolites.

29 **Biochemistry**

THE DISTRIBUTION OF CARBON-14 IN GLIOTOXIN FROM SERINE-1-C^{14} AND SERINE-3-C^{14}. R. J. Suhadolnik, A. Fischer, and J. Wilson (intr. by R. W. MacVicar) Dept. of Biochemistry , Okla. State Univ., Stillwater, Okla.

Earlier work established the origin of ten of the carbon atoms and one nitrogen atom of gliotoxin (Winstead and Suhadolnik, J. Am. Chem. Soc. (in press)). Structural considerations suggest that the three remaining carbon atoms and the other nitrogen might arise from serine. When serine-1-C^{14} and serine-3-C^{14} were added to growing cultures of Trichoderma viride, 2% and 3% respectively, of the C^{14} was incorporated into gliotoxin. The labeled gliotoxin was degraded to indole-2-carboxylic acid, to methylamine, and to 2-methyl-1,3,4-triketotetrahydropyrazino (1,2-a)-indole (MTP). The indole-2-carboxylic acid derived by degrading gliotoxin labeled from serine-1-C^{14} contained no isotope. Eighty-nine per cent of the radioactivity remained in the MTP, probably in C-4. Degradation of gliotoxin, derived from serine-3-C^{14}, by removal of the hydroxymethyl group (C-3a) resulted in MTP which contained 30% of the C^{14}; therefore, approximately 70% of the C^{14} from the hydroxymethyl group of serine appeared in the hydroxymethyl group of gliotoxin. These results suggest that serine can serve as a direct precursor for carbon atoms 3, 3a and 4 of gliotoxin. (Supported by a grant from the Atomic Energy Commission.)

30 **Biochemistry**

SYNTHESIS AND METABOLISM OF DL-TRYPTOPHAN-5-C^{14}. G. P. Mathur and L. M. Henderson. Dept. of Biochemistry, Okla. State Univ., Stillwater, Oklahoma.

DL-Tryptophan-5-C^{14} has been synthesized via benzoic acid-4-C^{14}. Ethyl formate was condensed with the Grignard reagent prepared from 1,5-dibromo-3-methylpentane to form 4-methylcyclohexanol-1-C^{14}. This was dehydrated and dehydrogenated simultaneously over palladium on alumina to toluene-4-C^{14}. The toluene was oxidized to benzoic acid-4-C^{14} in 15% yield based on $BaC^{14}O_3$. Benzoic acid-4-C^{14} was converted to aniline-4-C^{14} by Schmidt reaction. DL-Tryptophan-5-C^{14} was prepared from aniline-4-C^{14} by the procedure described earlier (Biochem. Prep. 6: 90, 1959). When DL-tryptophan-5-C^{14} (39.3 μc) was injected intraperitoneally to a rat 27.6% of C^{14} appeared in the expired CO_2 and 15% was excreted in the urine during 24 hour period. In a glutarate overloading experiment, the urinary glutarate was labeled. This confirms the earlier observation of Gholson, et. al. (Biochem. Biophys. Res. Comm. 1: 9, 1959) that glutarate is an intermediate in catabolism of benzene ring of tryptophan to CO_2. The labeling patterns of glutaric, glutamic and aspartic acids and alanine will be discussed.

31 **Biochemistry**

STUDIES ON THE ACTIVATION OF 3-HYDROXYANTHRANILIC ACID OXIDASE. R. H. Decker and F. R. Leach (intr. by W. D. Gallup) Dept. of Biochemistry, Okla. State Univ., Stillwater, Okla.

3-Hydroxyanthranilic acid has been identified as an intermediate in the metabolism of tryptophan by both the aliphatic and the niacin pathways. To elucidate the further intermediates on the two pathways and to establish unequivocally the structure of the product of 3-hydroxyanthranilic acid oxidation (compound I) it seemed desirable to purify the enzyme which catalyzes this oxidation. Stevens and Henderson (J. Biol. Chem. 234, 1188 (1959)) have previously demonstrated that glutathione and ferrous ions must be incubated with the enzyme at a low pH to obtain full activity with aged or dialyzed enzyme preparations. In attempting to purify further the enzyme by ammonium sulfate fractionation, it was found that all activity was lost. When ammonium sulfate (approximately 0.04 M) was added just prior to or during the assay of an active preparation, there was no effect on the enzymatic activity; however, if the ammonium sulfate were preincubated with the enzyme and other assay components for five minutes prior to the addition of substrate, an activation rather than an inhibition was observed. Since other salts also give this activation it appears that ionic strength is involved in the activation.

32 **Biochemistry**

METABOLITES OF LABELED S-METHYLCYSTEINE IN RAT URINE Edward J. Kuchinskas.(intr. by E.Muntwyler) Dept. of Biochem., S.U.N.Y., Downstate Med. Center, Bklyn,N.Y.

The metabolism of methyl labeled S-methylcysteine has been investigated in the rat (J.Biol.Chem. 234: 2935,1959). After multiple injections of the labeled compound, paper chromatography of rat urine in a pyridine-water solvent revealed three distinct radioactive areas with Rf's different from S-methylcysteine, its sulfoxides or sulfone. All three areas gave a positive platinic iodide reaction. Hydrolysis with 23% HI of the urine or eluates of the three areas resulted in a substance with the migration of S-methylcysteine which with dilute H_2O_2 treatment moved to the position of the sulfoxides. Eluates of one of the areas were ninhydrin negative which after 6N HCl hydrolysis gave ninhydrin reacting spots on rechromatography. Elucidation of the structures of the radioactive compounds is being pursued by the DNP and hydrolytic techniques. It is of interest that naturally occurring peptides of S-methylcysteine have been found in beans (Helv. Chim. Acta 41:1,1959; Arch. Biochem. Biophys. 73:281,1958). (Supported by a grant from the U.S. Public Health Service).

33 *Pharmacology*

STUDIES ON SEX DIFFERENCE IN METABOLISM OF HISTAMINE IN THE RAT. K.J. Netter* and P.A. Shore. Natl. Insts. of Health, Bethesda, Md.

A marked sex difference has been reported for the urinary excretion of free histamine by the albino rat, females generally excreting 5 to 10 times more histamine than do males. Blockade of diamine oxidase by aminoguanidine administration further elevates histamine excretion by females, but has little effect in males. These findings cannot be explained on the basis of a sex difference in the rate of histamine biosynthesis since a large fraction of injected histamine can be recovered unchanged in the urine of aminoguanidine treated females, but not males. It would appear that there should exist a clear sex difference in the activity in vitro of the other histamine metabolizing enzyme, imidazol-N-methyl transferase; however no sex difference could be demonstrated in the activity of this methylating enzyme in kidney, the richest source in the rat. Furthermore, there was no significant sex difference in the activity of liver methionine-activating enzyme or in intestine diamine oxidase. The lack of a sex difference in the enzymes measured in vitro, in spite of the marked differences in the whole animal suggests that other factors, permeability or renal, may have an important role in the metabolic fate.

34 *Pharmacology*

TRAUMA ON DISTRIBUTION IN MOUSE TISSUES OF S35 L-CYSTEINE. Lyle V. Beck, Sarah C. Kaiser* and Virginia R. Alexander.* Dept. of Physiology and Pharmacology, University of Pittsburgh School of Medicine, Pittsburgh 13, Pa.

In last year's report evidence was presented that tourniquet trauma inhibited incorporation of I.V. injected S35 l-cysteine into liver and kidney glutathione, isolated as the cuprous salt. In the present experiments, it was found that if a 45 rather than 90 minute distribution time were employed, this effect was much more striking and held, not only for liver and kidney, but also for intestinal mucosa and carcass (all other tissues, pooled together). Specific activities (CPM/mEq S) were estimated for liver glutathione by the isotope dilution method, and for dry liver protein. The protein sulfur determinations were made by combusting protein samples in a Schöniger flask and estimating the sulfate so formed by a modified barium chloranilate method. In the 5 experiments performed (2 control, 2 trauma, 1 adrenalectomy) the values obtained for liver for the ratio Glutathione specific activity/Protein specific activity ranged from 13 to 18. Protein samples isolated from intestinal mucosa exhibited much higher specific activities than did those isolated from liver, kidney, muscle or carcass. (Supported by NIH Grant.)

35 *Pharmacology*

CHANGES IN UTERINE FREE AMINO ACID PATTERN AFTER ESTROGEN TREATMENT. Sumner M. Kalman and Margaret E. Lombrozo*. Dept. of Pharmacology, Stanford Univ., Calif.

At periodic time intervals up to 30 hours after the intravenous injection of 2 microgms. of estradiol into ovariectomized rats the total free amino acid level in the uterus was estimated by reaction with ninhydrin. The free amino acid content per unit dry weight increased in a linear fashion from about 4 hours to 30 hours after hormone treatment; the increase amounted to 33% of the control value. During this same period the free amino acid content of the blood did not change perceptibly. Two-dimensional paper chromatograms of uterine amino acids were made for the 4 hour and 24 hour intervals after estrogen. These showed an increase in lysine, leucine, valine and alanine for the 4 hour period and a concomitant fall in the tissue glycine level. By 24 hours these displacements were not perceptible but glutamine had increased and the taurine level was depressed. It is concluded that estradiol increases the total free amino acid content of the uterus without changing the blood level, and that the increase is accompanied by an alteration in the amino acid pattern of this tissue. (Supported by USPHS Grant RG4632.)

36 *Pharmacology*

CONVERSION OF L-SERINE TO KETO ACIDS. Susan K. Fellner and Kenneth C. Leibman (intr. by T. H. Maren.) Dept. of Pharmacol. and Therap., Univ. of Florida Coll. of Med., Gainesville.

Mammalian enzymes are known to convert L-serine to keto acids by three pathways; formation of pyruvate by serine dehydrase (deaminase), of hydroxypyruvate (HP) by L-amino acid oxidase, and of HP by transamination with pyruvate. These reactions were studied in rat and dog liver with the aid of various inhibitors. In rat liver homogenates, HP (determined with HP reductase) accounted for less than 20% of total keto acids (determined with lactic dehydrogenase) formed from serine. Under aerobic conditions, isoserine reduced formation of both HP and total keto acids. In anaerobiasis, total keto acid formation was lower than in air, but was not diminished by isoserine; HP formation was the same as in air, and inhibited by isoserine. Keto acid production from serine was also diminished in homogenates by glyceric and diaminopropionic acids, glycine and ethanolamine. The transaminase and the dehydrase, purified from dog liver, were both inhibited by glyceric and diaminopropionic acids; the transaminase, but not the dehydrase, was inhibited by isoserine, glycine and ethanolamine. Inhibition of transaminase by isoserine was competitive. The results suggest that considerable keto acid other than HP is formed from serine by oxygen-requiring pathways.

37 *Pharmacology*

AMINE CONTENT AND BIOSYNTHESIS IN MASTOCYTOMAS AND OCTOPUS SALIVARY GLANDS. Wm. G. Clark, Med. Ctr., U. of Calif. & V.A. Ctr., Los Angeles, Calif.

Examination of the amine content of 4 mastocytomas, namely those transplantable in DBA/2, LAF/1 or AKD mice, and in dogs, has shown the presence of 5-hydroxytryptamine(5HT) and histamine(Hm). Evidence for the presence of 3,4-dihydroxyphenylethylamine(dopamine), m-tyrosine, 5-hydroxyindoleacetic acid and noradrenaline also has been obtained. Decarboxylation of histidine has been shown in all 4 tumors by radioactive methods. L-3,4-dihydroxyphenylalanine(dopa), DL-erythro-3,4-dihydroxyphenylserine(α-dops) and 5-hydroxytryptophan(5HTP) were decarboxylated, as determined by manometric and C^{14} methods. A similar investigation of the posterior salivary glands of Octopus hongkongiensis and O.bimaculata has shown the presence of such amines as 5HT, octonamine, dopamine and possibly m-tyramine; the decarboxylases producing them, and also those producing m-hydroxyphenylethanolamine and phenylethanolamine. The decarboxylase studies were determined manometrically, and the amine analyses were determined by paper and column chromatography and in some cases by chemical analysis. (Supported by grants from Am. Cancer Soc., Nat. Science Foundation and Calif. Inst. for Cancer Research, and USPHS.

38 *Nutrition*

PHENYLALANINE METABOLISM IN RATS FED RAW SOYBEAN OIL MEAL. Agaram Subba Rao* and Raymond Borchers. Dept. of Biochemistry and Nutrition, Univ. of Nebraska, Lincoln.

Rats fed raw soybean oil meal (SBOM) grow more slowly than rats fed heated SBOM. A recent report from our laboratory indicated that: 1) supplementation with methionine, threonine, valine, and tyrosine resulted in comparable rates of gain; and 2) phenylalanine would not replace tyrosine in this supplement. These results suggested that raw SBOM feeding interfered with phenylalanine metabolism. The present report on phenylalanine hydroxylase (PAH) supports this view. Liver PAH activity in rats on a raw SBOM ration for 3-4 days was reduced to 60% of that of animals on a heated SBOM ration. The level gradually increased to about 80% by the eleventh day. Endogeneous respiration, xanthine oxidase, and tyrosine oxidizing system activities were similar on the two rations. The apparent Km values for the PAH enzyme in livers of rats fed raw SBOM for more than 11 days, heated SBOM, or stock rations were of the same order—1.2×10^{-6}. However, the Km value from rats fed raw SBOM rations for 3-4 days appeared to be very large. (Supported by a grant from the National Institutes of Health.)

XUM

39 *Nutrition*

LEUCINE INDUCED HYPOGLYCEMIA IN CERTAIN CHILDREN. Francis J. Behal and Nancy Thornton. (intr. by Samuel A. Singal.) Depts. of Biochemistry and Pediatrics, Medical College of Georgia, Augusta, Georgia.

It has been reported that the ingestion of L-leucine by certain children has brought about a spontaneous idiopathic hypoglycemia (Cochrane et.al., J. Clin. Investigation, 35, 411 (1956)). In this hospital, another such patient has been treated. After only 30 minutes following the oral administration of L-leucine (150 mg./kg. body weight), the blood sugar value in this patient fell from 61 mg.% to as low as 24 mg.% on one occasion. L-valine and L-isoleucine have been found to produce the same response. Insulin activity assays (rat diaphragm technique) indicated that, among other things, leucine caused a rise of insulin or insulin-type activity in the patient's serum as soon as 30 minutes after the oral ingestion of the amino acid. Several lines of investigation have been followed in order to gain information about the mechanism of action of leucine. The results of these studies will be presented and discussed. (Supported in part by a grant from the Committee on Research of the Council of Drugs of the A. M. A.)

40 *Nutrition*

1,2-C^{14}-AMINOETHANOL METABOLISM IN THE CHICK. G. J. Klain* and B. Connor Johnson. Dept. of Animal Science, Univ. of Illinois, Urbana.

The metabolism of radio-aminoethanol has been studied with regard to its conversion to choline, glycine and serine in the animal body and with regard to its metabolic excretion products. Chicks weighing 400 to 450 gm. were given 50 μc. of 1,2-C^{14}-aminoethanol in four separate experiments. The chicks were operated on so that urine uncontaminated with feces could be obtained. The urine was chromatographed on paper and the chromatograms were counted on a strip counter. Uric acid was recrystallized and counted. The chicks were killed at varying time intervals from 4 to 48 hours. The carcasses were solvent-extracted and the liver proteins were hydrolyzed, glycine, serine, aminoethanol and choline being isolated following separation on Dowex columns. The results show considerable conversion of aminoethanol to choline in the body, but no labeling of tissue glycine or serine even after 48 hours. On the other hand, 9% of the radioactivity administered was secreted in the urinary uric acid and somewhat less in the urinary creatine and creatinine after 24 hours, during which 16% of the C^{14} appeared as CO_2. These data indicate that free glycine is not an intermediate between aminoethanol and uric acid. Data will also be reported on comparable experiments with radioglycine.

41 *Nutrition*

THE TREATMENT OF A CASE OF MAPLE SYRUP URINE DISEASE. L. E. Holt, Jr.; Selma E. Snyderman; Joseph Dancis; and Patricia M. Norton*. Department of Pediatrics, New York University College of Medicine, New York.

This disease is characterized clinically by the excretion of urine having an odor similar to that of maple syrup, by severe mental retardation and a variety of neurologic disturbances. There are abnormally high levels of leucine, isoleucine, valine and methionine in the blood and urine and large amounts of the keto acid analogues of these amino acids are also present. The exact site of the metabolic block is as yet unknown. An attempt was made to treat a two week old infant with this disease by means of a completely synthetic diet in which the intake of these four amino acids could be carefully controlled. With this regime, it has been possible to control the blood levels of the branched chain amino acids; however, the methionine level has remained high in spite of extremely low intake. At present, this infant is seven months old and appears neurologically and mentally normal.

42 *Nutrition*

EARLY UPTAKE OF DL-METHIONINE-2-C^{14} AND L-METHIONINE-METHYL-C^{14} BY RAT TISSUES. Cecile H. Edwards, Evelyn L. Gadsden, and Gerald A. Edwards (intr. by Pearl P. Swanson.) Departments of Home Economics and Chemistry, The Agricultural and Technical College of North Carolina, Greensboro, North Carolina.

Adult rats of Wistar strain were fed dl-methionine-2-C^{14} (R11 sacrificed at 16 minutes) or l-methionine-methyl-C^{14} (R12 sacrificed at 20 minutes; R13, 1440 minutes) by stomach tube following a 10 hour fast. The specific activities of fresh tissues and their water-soluble, fat-soluble and protein components were determined. Of 18 water-soluble fractions of R11, pancreas was most active, followed by adrenals, thymus, kidney and liver in decreasing order; whereas, liver was most active in R12, followed by adrenals, kidney, pancreas and spleen. The fat-soluble fraction of the liver of R12 was 40 times more active than the next most active tissues of R11 and R12. All protein fractions were inactive for R12, while liver, pancreas, spleen and kidney, in decreasing order, showed activity in R11. The pattern of radioactivity had changed considerably at 1440 minutes, with bone marrow, adrenals and pancreas containing the most active water-soluble, fat-soluble and protein fractions, respectively. Preliminary studies on the mode of transport of methionine in the blood will be presented. (Supported in part by a grant, A-1464, from the National Institutes of Health, Public Health Service.)

43 *Immunology*

HISTIDINE DECARBOXYLASE IN LEUKEMIC BASOPHILS. William J. Hartman (Intro. by Raymond L. Libby). V.A. Center and Univ. Calif. Med. Ctr., Los Angeles.

Buffy layers from blood of two patients with chronic myelogenous leukemia and high basophilia were examined for histidine decarboxylase content. The cells were collected in a solution of citrate and polyvinylpyrrolidone, centrifuged, washed in saline, resuspended in distilled water, and incubated for 3 hrs. under sterile conditions with l-histidine-2(ring)-C^{14} in glucose-phosphate buffer, pH 7.4 in the presence of pyridoxal phosphate and aminoguanidine. 0.1ml of wet, packed cells and 200,000 cpm of labelled histidine were used in each reaction vessel. Repeated recrystallization of the histamine picrate yielded constant activities of the order of 400-700 cpm as compared with killed controls of 40 cpm(background), thus demonstrating the presence of mammalian histidine decarboxylase in the absence of bacteria. Control experiments under these exact conditions were performed on the buffy layers of patients with chronic lymphatic and acute myelogenous leukemia (98% lymphocytes and 98% myeloblasts, respectively), with negative results. (Supported by Am. Cancer Soc., and California Institute for Cancer Research).

005. AMINO ACIDS–Nutrition

1 *Physiology*

THE EFFECT OF LYSINE ON DENTAL CARIES AND GROWTH IN THE RAT. Eleanor J. Edmonds*, Kenneth O. Madsen*, and Edwin L. Smith, University of Texas Dental Branch, Houston, Texas

McClure et al. reported increased smooth surface caries and a severe growth depression in albino rats when the skim milk powder of the cariogenic diet was autoclaved. Supplementation with L-lysine improved growth and decreased caries. Growth was greatly impaired by the autoclaved-milk diet even in the presence of added lysine. In the present experiment, the milk diets were prepared as McClure did. However, the autoclaved-milk did not depress growth, nor increase caries beyond that obtained on the unautoclaved diet. When the autoclaved-milk diet was supplemented with lysine, there was a growth stimulation and a reduction in dental caries in both sexes. The lysine growth response was greater in the males and optimal compared to the growth obtained on a stock diet. A response to added lysine was also seen in those animals fed the unautoclaved-milk diet; only the males showed a growth response while only the females had decreased caries. The data showed that lysine supplementation decreased dental caries even at a level of nutrition which supported good growth. The effect of heating on the nutritional value of skim milk powders was emphasized by the fact that greater growth was obtained when the lysine-supplemented diet contained autoclaved milk than when it contained unautoclaved milk. Support - N.I.H. Grant D-1063.

2 *Biochemistry*

OBSERVATIONS ON LEUCINE-ISOLEUCINE ANTAGONISM IN THE RAT. Pari D. Spolter* and A. E. Harper. Dept. of Biochemistry, Univ. of Wisconsin, Madison.

Addition of 3% of L-leucine to a diet containing 9% of casein causes retardation of the growth of rats which can be prevented by a further supplement of isoleucine (Harper et al. Arch. Biochem. Biophys. 57: 1, 1955). Rats adapt within 3-5 days to diets containing 3% of L-leucine, so to prolong the time during which the mechanism of the antagonism can be studied, the effects of higher dietary levels of leucine have been tested. Increasing the level of L-leucine added to the diet from 3 to 5 or 7% caused a progressive increase in the severity of the growth retardation and prolonged the time required for adaptation to as much as 15 days. The growth depression caused by 5% of L-leucine, was only partially prevented by isoleucine but was almost completely prevented by a combination of L-isoleucine and L-valine (0.16% of each). Stimulation of food intake by insulin injections also reduced the severity of the antagonism. Results of nitrogen balance studies and of experiments with diets in which amino acids were substituted for casein indicate that the effect of leucine is not a result of inhibition of protein digestion or overall nitrogen absorption. The effect of excess leucine on blood amino acid pattern is being studied.

3 *Biochemistry*

METHIONINE REQUIREMENTS OF MEN OVER SIXTY. Stewart G. Tuttle*, Marian E. Swendseid and Samuel H. Bassett*. Veterans Administration and Univ. of Calif., Los Angeles.

This study was designed to determine the methionine requirements of men over sixty using the nitrogen balance technique. Six male subjects were placed on control diets of ordinary food. The caloric content was varied according to individual requirements, but protein intakes were maintained at 7 grams of nitrogen. When nitrogen equilibrium was established, the subjects were given isonitrogenous semi-synthetic test rations containing mixtures of purified essential amino acids in at least double the quantities found necessary to maintain young adults in nitrogen equilibrium (Metabolism 6: 564, 1957). Supplementary non-essential nitrogen was supplied as a mixture of purified non-essential amino acids in the ratio found in egg protein. These diets were alternated with the control diets at 8 to 10 day intervals. Three grams of methionine per day were given initially in the test diets and then gradually reduced until a negative nitrogen balance occurred. Under these experimental conditions, it was found that the methionine requirements of these older individuals ranged from 2.4 to 3.0 grams per day, more than double the needs reported by Rose for young men. (Supported in part by USPHS grant A-2323 and by Nutrition Foundation Inc.)

4 *Pathology*

SIMILARITY OF PANCREATIC LESIONS PRODUCED BY EXCESSIVE AMOUNTS OF METHIONINE AND BY ETHIONINE. Janis V. Klavins*, Nathan Kaufman, and Thomas D. Kinney. Cleve. Met. Gen. Hosp. and Western Reserve University, Cleveland, Ohio

Male albino rats were fed synthetic diets containing 18% protein in the form of casein for 30 days. One group of rats received a 2% and another a 4% methionine supplement. Controls were pair fed for each group. Additional controls consisted of animals fed ad libitum and also animals on a decreased dietary intake so as to produce a weight loss equivalent to or in excess of that in the experimentals. The damage to the acinar tissue of the pancreas in rats fed purified diets supplemented with methionine was a striking and constant finding in all of the experimental animals. There was loss of basophilia, vacuolization of the cytoplasm and pyknosis of nuclei of acinar cells as well as degeneration and loss of individual acinar cells. Regeneration of acinar cells also was found. These changes were similar in all respects to those found in the pancreas following the administration of ethionine for a similar period of time. (Supported by U.S.P.H. Service Grant #A-1388.)

5 *Nutrition*

QUANTITATIVE ASPECTS OF AMINO ACID IMBALANCE. Hans Fisher, P. Griminger and G.A. Leveille*. Rutgers Univ., New Brunswick, N. J.

The present studies were undertaken to obtain quantitative information on the factors leading to amino acid imbalance, following the suggestion of Harper (J. Nutr., 68:405, 1959). Three series of experiments were conducted with week-old chicks. In the first series protein and lysine levels were varied and a mixture of amino acids devoid of lysine but otherwise balanced, added to produce an imbalance. Such an imbalance occurred at high and low protein levels on an intermediary lysine concentration. No growth depression was observed on severely deficient or on adequate lysine levels. In the second series of experiments it was shown that: 1) An imbalance occurs only when sufficient lysine above the maintenance requirement but inadequate for optimum growth is present, 2) The % growth depression is equal on low and high protein intakes provided the nitrogen supplementation used to produce the imbalance is added in comparable amounts as a percentage of the total protein level and 3) The growth depression resulting from the imbalance is primarily a reflection of a changed amino acid (lysine):protein relationship. The third series of experiments suggested that considerable specificity for the nitrogen supplement is necessary to induce an imbalance.

6 *Nutrition*

ISOLEUCINE, LEUCINE, PHENYLALANINE AND VALINE REQUIREMENTS OF CHINOOK SALMON. R.E. CHANCE*, J.E. HALVER and E.T. MERTZ. Western Fish Nutr. Lab. USFWS, Cook, Wash., and Dept. Biochem., Purdue Univ., Lafayette, Ind.

Quantitative amino acid requirements of Chinook Salmon (Oncorhynchus tshawytscha) for lysine, threonine, methionine and cystine have been reported (Fed. Proc. 17: 478, 1948; 18: 527, 1959). To study the requirements for isoleucine, leucine phenylalanine and valine, salmon fingerlings were fed 40% protein diets composed of casein, gelatin, amino acids, dextrin, corn oil, vitamins, minerals, α-cellulose and water. The diets contained a balance of indispensable amino acids similar to whole egg protein with exception of increasing increments of those specific amino acids studied. For isoleucine, 250 fish av wt 1.0 gm were used per trough. Results indicated a requirement of approx. 1.0% of the dry diet. An iso:leu ratio of 1.0:1.5-2.0 gave satisfactory growth but high leu in the diet (ratio 1.0:6.0) depressed growth and feed conversion. Fish av wt 2.5 gm at 200 per trough were used to estimate leu, phe, and val needs. Overall performances indicated approx. requirements were: leu 1.5%; phe 2.0%; and val 1.5% of dry diet.

7 *Nutrition*

INDISPENSABLE AMINO ACIDS FOR SOCKEYE SALMON AND RAINBOW TROUT. W. E. Shanks* and J. E. Halver. Western Fish Nutr. Lab. USFWS, Cook, Washington.

Specific amino acid deficient diets were prepared by deleting one amino acid and replacing it with α-cellulose flour from the basal ration consisting of 17 L-amino acids as the nitrogen source. Other dietary components were maintained constant as in previous studies with salmon (Fed. Proc., 15, #1803, 1956). Growth of 20 lots of sockeye salmon (Oncorhynchus nerka) fingerlings and rainbow trout (Salmo gairdneri) fed these diets were compared with that obtained with the complete basal ration. From these trials sockeye salmon required arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine for normal growth. No partial biosynthesis of any of these amino acids was observed. In contrast, alanine, aspartic acid, cystine, glutamic acid, glycine, proline and tyrosine were not required for growth and may be considered dispensable under the experimental conditions used. By inspection hydroxyproline and serine can also be so classified. Similar results were obtained with rainbow trout under comparable experimental conditions.

XUM

8 *Nutrition*

STUDIES ON A SHORT TERM PROCEDURE FOR DETERMINING AMINO ACID REQUIREMENTS OF LAYING HENS. Edward C.Miller,*Jean S. O'Barr,* and Charles A. Denton. U.S. Dept. of Agriculture, Animal Husbandry Research Division

Studies were made on a short-term procedure for determining amino acid requirements of laying hens utilizing radioactive tracers. SCW Leghorn pullets from the same hatch were used in all studies. The pullets were kept in laying batteries and maintained under identical conditions in a constant temperature room. A long-term study (8 month) using the same dietary treatments as used for the short-term study was conducted in order to evaluate the results of the short-term procedure. Results of the long-term study indicated that the basal diet was deficient in lysine. The short-term studies using the radioactive tracer procedure did not detect the lysine deficiency in the basal diet.

In the short-term studies it was necessary to restrict the feed from 20 to 30% of that of the ad libitum fed group before a dietary deficiency was detected by the radioactive tracer procedure. However, at 10% level of feed restriction losses in body weight occurred. At 20% level of feed restriction greater loss in body weight and a reduction in egg production resulted.

9 *Nutrition*

THE IMPORTANCE OF DISPENSABLE AMINO ACIDS FOR NORMAL GROWTH OF CHICKS. W. P. Stucki,* A. E. Harper and C. A. Elvehjem. Dept. of Biochemistry, Univ. of Wisconsin, Madison.

The observation of Benton et al. (Arch. Biochem. Biophys. 70: 491, 1957) that a supplement of 5-10% of gelatin stimulates the growth of chicks fed a diet in which crystalline amino acids have been substituted for protein has been reinvestigated and a possible explanation for this effect has been obtained. Chicks were fed a basal diet containing 23.4% of an amino acid mixture which supplied 1.5 times the accepted requirement for each of the indispensable amino acids and at least 0.5% of each of the common dispensable amino acids. The experimental period was 10 days. The basal diet supported a growth rate of only 3 to 5 gm/day but the rate was doubled when the diet was supplemented with 5% of gelatin. Supplements of individual amino acids or mixtures of amino acids were generally ineffective in stimulating growth, but 5% of a 1:1:3 mixture of glycine, proline and glutamic acid produced a growth response equivalent to that obtained with 5% of gelatin. Further work has shown that a relatively high proportion of dispensable amino acids is essential for optimal growth of chicks and that there is a significant interaction between the dietary level of nitrogen and the ratio of dispensable to indispensable amino acids.

10 *Nutrition*

SOME FURTHER OBSERVATIONS ON AMINO ACID IMBALANCE. U. S. Kumta* and A. E. Harper. Dept. of Biochemistry, Univ. of Wisconsin, Madison.

Addition of 0.5% of a mixture of methionine and phenylalanine, the second most limiting amino acids in fibrin, to diets containing 6% of fibrin causes depressions in growth, nitrogen retention and food intake of young rats. These effects can be prevented by the further addition of 0.25% to 1% of a mixture of the most limiting amino acids in fibrin, viz. Leu, Iso-leu, Val, and His. [Harper, A. E. and Kumta, U. S. Fed. Proc. 18: 12, 1959]. An effort was therefore made to determine how specific this effect was for the second most limiting amino acids. Among the amino acid combinations tested, mixtures of the least limiting amino acids tryptophan, arginine and threonine also caused growth depressions. These too were prevented by a supplement of 0.5% of the most limiting amino acids, but not by a variety of other amino acid mixtures. Blood urea levels were determined in protein-depleted rats at various time intervals after they had been fed for 2 hours on the diets used in the growth studies. A significant increase in blood urea occurred when the diets contained methionine and phenylalanine or mixtures of the least limiting amino acids but not when they contained several other amino acid mixtures.

11 *Nutrition*

COMPARISON OF ESSENTIAL AMINO ACID PATTERNS. Johnnie H. Watts, Marian E. Swendseid, Cheryl L. Harris and Stewart G. Tuttle (introduced by Helen G. Oldham). University of California, Los Angeles.

The response of young men to diets containing essential amino acids proportioned as in (1) a reference pattern based on human requirements and (2) the pattern present in whole egg was determined by nitrogen balances. Amounts of each essential amino acid in the diets for each man were based upon the amount of tryptophan which was found to maintain nitrogen equilibrium or a slightly negative nitrogen balance. Six men were studied on diets containing each pattern when (1) the chief source of essential amino acids was a purified amino acid mixture and (2) the chief source of essential amino acids was whole egg. Other sources of nitrogen were purified non-essential amino acids and diammonium citrate. The total nitrogen intake was always 10.0 gm., and caloric intakes were 50-55 Cal/kgm. As judged by nitrogen balances, the response of 5 subjects to the egg pattern was better than to the reference pattern. This response was observed both when the source of essential amino acids was a purified mixture and when whole egg was fed. (Supported by a USDA contract sponsored by the Human Nutrition Research Division, Agricultural Research Service.)

12 *Nutrition*

AMINO ACID INTERRELATIONSHIPS. Selma E. Snyderman; L. Emmett Holt, Jr.; Patricia Norton*; and Ellen Roitman*. Department of Pediatrics, New York University College of Medicine, New York, New York.

The quantitative determination of plasma amino acids during periods of amino acid withdrawal and loading has revealed some interesting interrelationships between amino acids. Withdrawal of isoleucine resulted not only in a reduction of the plasma isoleucine level but also in a striking increase in the levels of the aromatic amino acids. Tyrosine was increased 9 to 19-fold and there was a 2 to 3-fold increase in the phenylalanine. The converse occurred after an oral load of isoleucine; tyrosine and phenylalanine were both depressed, but to a lesser degree. Both withdrawal and loading with methionine resulted in similar changes in certain plasma amino acids; there was a significant fall in the levels of the branched chain amino acids. Leucine fell 30 to 40 percent, isoleucine 40 to 70 percent, and valine 25 to 60 percent. In addition, an oral load of methionine also produced a 70 percent fall in tyrosine and a 50 percent reduction in the phenylalanine level. These variations in plasma amino acids suggest the existence of previously unrecognized metabolic interrelationships.

13 *Nutrition*

EFFECTS OF ADDED AMINO ACIDS OR SUGARS ON THE PATTERN OF ABSORPTION OF AMINO ACIDS FROM AN ISOLATED ILEAL LOOP IN MAN. Aline U. Orten, N. S. Gimbel*, and A. H. Smith, Wayne State University College of Medicine, Detroit.

Our previous studies with an equimolar mixture of 18 amino acids have shown that these amino acids are absorbed in a characteristic pattern. They can be divided into 3 groups: (1) absorbed rapidly (isoleucine, methionine, arginine, leucine); (2) absorbed at an intermediate rate (valine, lysine, phenylalanine, cystine, tyrosine, aspartic acid, proline, tryptophan, alanine, serine); (3) absorbed slowly (threonine, histidine, glycine, glutamic acid). Increased amounts of certain individual amino acids or the addition of sugars alters the total absorption but not the pattern; whereas other amino acids affect the pattern as well as the total absorption. (Supported by Grant A-693 from the National Institutes of Health).

14 *Nutrition*

CLINICAL TRIALS OF WATER-SOLUBLE CHEMICALLY DEFINED DIETS. R.B. Couch*, D.M. Watkin, R.R. Smith*, L.E. Rosenberg*, M. Winitz, S.M. Birnbaum, M.C. Otey*, and J.P. Greenstein. Natl. Insts. Health, Bethesda, Maryland.

The clinical utility of water-soluble, chemically defined diets containing all known nutrients required by man has been evaluated in patients with slowly progressing neoplasms. Preliminary studies revealed that the diets maintained body weight and normal organ function. Definitive metabolic studies were carried out in 2 patients using a diet containing L-amino acids essential for man. In one patient a diet in which DL-isomers were substituted for more costly L-isomers was also tested. The diets contained daily .16 g. N/kg. body weight and 40 or 50 cal./kg. An isonitrogenous blended natural food diet supplying 40 cal./kg. was the control regimen. N balances were positive on all diets but could be ranked in terms of highest to lowest positive N balance as follows: control diet, 50 cal./kg. L-isomer diet, 40 cal./kg. L-isomer diet, and 40 cal./kg. DL-isomer diet. A second patient, depleted of N during 12 days on an isocaloric N-free soluble diet, stored 2.9 g. N/day during repletion with the experimental diet. Clinical complications were negligible. The data indicate that chemically defined diets will maintain positive N balance and permit N repletion in adult man.

15 *Nutrition*

NITROGEN BALANCE RESPONSE OF YOUNG MEN TO CHANGES IN QUALITY OF DIETARY PROTEIN. H.L. Rice* and N.W. Flodin, Lysine Div., E.I. du Pont de Nemours & Co., Inc., Wilmington, Del., and A.C. Shuman*, Shuman Chemical Laboratory, Lafayette, Ind.

N balance studies were carried out with 15 male grad. college students consuming diets in which 95% of the protein came from enriched bread made with 4% nonfat dry milk. Diets were fed at 2 protein intakes: 0.7 g/kg and 1.0 g/kg of body wt. Standardization did not involve depletion by a low-protein or essen. amino acid-free diet, but was done by feeding the bread diets for 9 days, during which the lysine intakes, entirely from food protein, were at 180-225% of the min. requiremt. for N equ. as detd. by Rose. N bal. responses were then measured in succeeding 9-12 day periods on lysine-suppd. bread diets, while control groups continued on the same diets without added lysine. Levels of 0.15, 0.25, and 0.45% L-lysine·HCl, based on flour wt., were baked into the bread, incorporation being done isonitrogenously at expense of glycine in the standardizing diet. Group responses to lysine supplementation were pos. and statist. significant at both 0.7 and 1.0 g/kg protein intakes. The results are consistent with the interpretation that, at normal protein intakes providing essen. amino acids at levels well in excess of min. requiremts., the level of labile protein stores in the normal adult rises as the quality of dietary protein increases.

006. ATHEROSCLEROSIS

1 *Physiology*

RELEASE OF HEPARIN-INDUCED LIPEMIA CLEARING FACTOR FROM PERFUSED RAT HEART. M. F. Crass*, III and H. C. Meng, Physiol., Vanderbilt, Nashville.

The mechanism of production of lipemia clearing factor following intravenous administration of heparin remains obscure. The present study was to investigate the release of this factor from an isolated rat heart. Hearts removed from male rats were perfused with bicarbonate buffer (pH 7.4) containing various concentrations of rat serum or human serum albumin in the presence of heparin. Perfusates were withdrawn at intervals to determine in vitro clearing activity which was measured by the decrease in optical density of a cottonseed oil emulsion and the increase in free fatty acid concentration. The following results were obtained: 1. No clearing activity appeared in the perfusates in the absence of heparin and/or serum component(s). 2. Optimal conditions for the release of clearing factor were the presence of 5% serum and 3-10 mg% of heparin. 3. Human serum albumin (250 mg%) could replace rat serum. 4. The highest clearing was obtained after 30-40 min. perfusion. Further studies are in progress to identify the serum component(s) required and the role of heparin in the release of heart clearing factor.

2 *Physiology*

PRELIMINARY STUDY OF LIPEMIA CLEARING DEFECT IN ALLOXAN DIABETIC RAT. R. J. Ho and H. C. Meng (intr. by P. F. Hahn). Physiol., Vanderbilt, Nashville.

It is known that the clearing activity of postheparin plasma of alloxan diabetic rats was considerably reduced. The present investigation was to determine the mechanism(s) of this defect. We have found that the clearing activity of postheparin plasma of alloxan diabetic rats was increased to that of normal animals when human serum albumin was added to the in vitro clearing system, except the slow rate of clearing during the early phase of the reaction. The increase in clearing was directly proportional to the amounts of serum albumin added up to 1.5 gm% above which no further increase was observed. It was further found that the average free fatty acid concentration of postheparin plasma of alloxan diabetic rats was 3.56 mEq/L, while that of normal rats was 1.22 mEq/L. Thus it is possible that the reduced clearing of postheparin plasma of alloxan diabetic rats is in part due to its high concentration of free fatty acid, which "saturates" serum albumin rendering it ineffective as a fatty acid acceptor during in vitro clearing.

3 *Physiology*

EFFECT OF VAGUS STIMULATION ON BLOOD LIPID LEVELS IN THE BABOON. - (1) THE EFFECT ON CHOLESTEROL ESTER LEVELS. N. T. Werthessen. Southwest Foundation for Research and Education, San Antonio, Texas

Experiments employing baboons prepared by ligation of the carotid artery led to the discovery that in some way the vagus nerves are involved in the maintenance of lipid levels of the blood. The right and left vagi have been stimulated mechanically and electrically both when intact and severed in 16 large adult baboons. The data presently available show that if blood is collected from a peripheral vein, while the intact right vagus nerve is stimulated to the point of inducing cardiac arrest, significantly large drops in cholesterol ester levels can be seen. (Reductions from a control level of 64 mg.% to 38 mg.% have been seen to occur during the period of stimulus with recovery to normal within 5 minutes. The free cholesterol level remains constant (± 2 mg%) thruout.) Stimulation of the left vagus nerve shows no effect. The data at hand are only sufficient to show that the vagus nerves can affect cholesterol ester levels. They are insufficient to indicate the modus operandi.

Changes in the total fatty acid levels seen in these baboons will be reported by Baeder and Seifter.

4 *Biochemistry*

ON THE SITE OF THROMBIN TOPICAL INHIBITION OF THE LIPOPROTEIN LIPASE (LPL) SYSTEM. L. O. Pilgeram. Arteriosclerosis Research Lab., St. Barnabas Hosp. Research Found., and Dept. of Physiology, Univ. of Minnesota, Minneapolis.

Thrombin inhibition of the LPL system has been reported (Pilgeram and Tu, J. Applied Physiol. 11:450, 1957). The purpose of this investigation was to clarify the nature and site of the inhibition phenomenon. LPL has little or no capacity for removing thrombin inhibition whereas comparable concentrations of serum prevent thrombin inhibition and increase LPL system activity. Partial destruction of serum antithrombin by preincubation with small quantities of thrombin does not impair the capacity of that serum to provide LPL cofactor activity. However, preincubation of LPL with similar quantities of thrombin completely destroys LPL activity. It is concluded that thrombin exerts a part of its inhibitory action on the LPL system by direct destruction of LPL. However, this observation does not preclude the possible function of antithrombin as a cofactor in the LPL system. Serum antithrombin and LPL cofactor activity possess certain degrees of similarity in that both are removed from serum by $BaCO_3$ and $Al(OH)_3$ gels, both largely destroyed by heating at 70° for short periods, and both destroyed by ether extraction. (Supported by N.I.H., Amer. and Minn. Heart Assoc., Life Insurance Med. Research fund, and Graduate School, Univ. of Minn.)

XUM

5 *Pharmacology*

THE EFFECT OF TRIPARANOL (M/E/R/29®) ON EXCRETORY PATHWAYS OF STEROLS. Takashi Kariya and Thomas R. Blohm (intr. by E. F. Van Maanen), Department of Biochemistry, Scientific Division, The Wm. S. Merrell Company, Cincinnati, Ohio.

Previous work (Arch. Biochem. 85, 245, 250, 1959) showed that triparanol inhibits cholesterol biosynthesis in the rat at a late stage in the biochemical pathway. This action could lead to accumulation of unsaponifiable intermediates in the tissues, but such accumulation was found to be less than the deficit in cholesterol. The fate of the intermediates was investigated by following excretion of radioactivity in fecal bile acids and unsaponifiable matter following administration of d,l-mevalonate-2-C^{14} intraperitoneally to rats treated with triparanol. Over a ten-day period, the triparanol rats excreted a greater fraction of administered radioactivity in these forms than controls. This was attributed to reduction of the pool of labeled sterols and other intermediates by triparanol, plus the fact that the end products of the sterol-synthetic pathway in triparanol-treated rats are excreted as unsaponifiable matter and acidic products, probably bile acids.

6 *Pharmacology*

DISTRIBUTION AND EXCRETION OF RADIOACTIVE TRIPARANOL (M/E/R/29®) IN THE ALBINO RAT. Robert D. MacKenzie and Thomas R. Blohm (intr. by E. F. Van Maanen), Department of Biochemistry, Scientific Division, The Wm. S. Merrell Company, Cincinnati, Ohio.

Triparanol has previously been reported to lower the cholesterol level in the blood by inhibiting its biosynthesis (Arch. Biochem. 85, 245, 1959). Radioactive triparanol labeled in the carbinol position with C-14, was given by stomach tube to male albino rats. The rats had received unlabeled triparanol for ten days before the radioactive material was given. Urine and feces were collected for two days. The liver, lung, stomach, kidney, spleen, heart, brain, testis, and fat contained about 5% of the injected dose. Another 5% was found in the intestine. The greatest excretion of radioactivity was in the feces, only about 7% was excreted in the urine. In another experiment in which rats were given radioactive triparanol without previous dosing, the same general pattern was evident. No significant count was found in CO_2. When bile was collected, it contained considerable radioactivity. Urine and bile were tested to determine whether the radioactive material was triparanol. The data indicated that triparanol is mainly excreted in the feces via the bile, but that most of it has been chemically modified before excretion. Very little of it remains in the main organs two days after ingestion.

7 *Pharmacology*

CHRONIC TOXICOLOGICAL AND PATHOLOGICAL STUDIES WITH TRIPARANOL (M/E/R/29®). William M. King and J. Knox Smith (intr. by E. F. Van Maanen), Department of Toxicology-Pathology, Scientific Division, The Wm. S. Merrell Company, Cincinnati, Ohio.

The acute, subacute, and chronic toxicity of triparanol was investigated in mice, rats, dogs, and monkeys. The acute toxicity of the insoluble triparanol base was 1600 ± 132 mg/kg in the mouse and in excess of 2000 mg/kg in the rat. Triparanol appears to be slightly more toxic in the male than in the female. The minimal effective dose of triparanol approximated 2 mg/kg/day. In the male rat 50 mg/kg/day was well tolerated and in the female, 35 mg/kg/day was well tolerated. Therefore, the spread between the minimal effective dose and the maximal tolerated dose provides a comfortable margin of safety. No untoward hematopoietic response was demonstrated in the rat, dog, or monkey. Triparanol did not affect measurable liver function in the monkey following long-term administration of doses of 20 mg/kg/day. A consistent change was seen in the adrenals of all the species, and this change consisted of a partial lipoid depletion in the cells of the zona fasciculata (spongiocytes). However, no changes were seen in the overall architecture of the organ. No other gross or microscopic pathology was demonstrated in any tissues.

8 *Pharmacology*

THE EFFECT OF VAGUS STIMULATION ON BLOOD LIPID LEVELS IN THE BABOON. 2. THE EFFECT ON TOTAL FATTY ACIDS. David H. Baeder and Joseph Seifter. Wyeth Institute for Medical Research, Radnor, Pa.

The observation was made that manipulation of the vagus nerve in baboons decreased the serum lipid levels. Studies carried out in adult baboons anesthetized with 1-2 mg/kg Sernyl® I.M. demonstrated that mechanical or electrical stimulation of the intact right vagus resulted in a marked lowering of total fatty acids as measured by the method of Stoddard and Drury. Stimulation of the intact left vagus resulted in variable changes in total fatty acid levels. Stimulation of the distal portion of the cut right vagus resulted in lowering of total fatty acids. Stimulation of the central portion of the cut left vagus also caused lowering of total fatty acids. Work to elucidate the physiological significance of these observations is in progress. Changes in the cholesterol esters seen in these baboons will be reported by Werthessen.

9 *Pathology*

AORTIC QO_2 OF NORMAL, CHOLESTEROL-FED, HYPERTENSIVE AND CORTISONE TREATED RABBITS. J.H. Geller,* Edwin R. Fisher and E. Tapper. Univ. of Pgh. and VA Hospital, Pittsburgh, Pa..

Interest in the metabolic activity of the aorta and its possible relationship to the pathogenesis of atherosclerosis warranted investigation of the oxygen consumption of the aortas from rabbits 1) maintained on a stock laboratory diet (controls). 2) with cholesterol atherosclerosis, 3) with induced renal hypertension, 4) following prolonged administration of cortisone, 5) with hypertension and atherosclerosis, 6) receiving cortisone and cholesterol, and 7)receiving cortisone with hypertension and atherosclerosis. QO_2 was determined for the arch, thoracic and abdominal portions of aorta in all instances by the standard Warburg manometric technic utilizing Krebs-Ringers solution. In all situations the QO_2 was greatest in abdominal and least in thoracic segments. The hypertensive state, cortisone administration and their combination with or without cholesterol atherosclerosis resulted in a marked elevation of QO_2 whereas all aortic segments from rabbits with cholesterol atherosclerosis and cholesterol-fed rabbits receiving cortisone disclosed values less than or approximating those observed in controls. Although these findings do not preclude the possible importance of such activity in the pathogenesis of atherosclerosis their lack of correlation with the sites of predilection for this disorder and the degrees of atherosclerosis observed under these various experimental conditions minimizes the primary significance of the aortic QO_2 in this regard.

10 *Pathology*

ATHEROSCLEROSIS IN ST. LOUIS: A COMPARATIVE GROSS STUDY OF AORTAS. A.A.Dimakulangan*, J.Owen Blache* and R.M.O'Neal, Washington University, St. Louis, Mo.

Aortas from adult autopsied patients were fixed in formalin and stored in transparent plastic bags. Grading of degree of atherosclerosis was accomplished by placing a sheet of plexiglass, marked into cm. squares, over the aorta. Total area of the aorta from the superior mesenteric artery to the bifurcation was calculated, and the abnormal squares were counted. Abnormal divided by total area gave percentage involvement. Percentages of involvement by mural thrombosis, ulceration and calcification were also determined. 140 Negroes and 148 Whites, matched for sex and decade of life were available for comparison. No significant differences were found between the Whites and Negroes in percentage of aorta abnormal, but in white males the percentages were greater ($p<0.05$) than in white females. The greatest differences found involved ulcerated lesions, greater ($p<0.01$) in Whites than Negroes for both sexes. Calcified lesions also involved more of the aorta in Whites than Negroes ($p<0.05$). Percentages of involvement by thrombotic lesions were almost identical in the two races and sexes. The difference in ulcerated lesions contrasts with the lack of difference in "degree" of atherosclerosis between the two races, and suggests that size of the lesion does not determine the likelihood of ulceration. (Supported by a grant from the National Heart Institute.)

11 *Pathology*

DIETARY Mg-PRIMING EFFECT ON RAT HEART VALVE-AORTA LIPIDOSIS. E. E. Hellerstein, M. Nakamura*, D. M. Hegsted and J. J. Vitale. Harvard Medical School (Path.) and Harvard School of Public Health (Nutr.), Boston, Mass.

Expt. I. Male rats (60 gm) were fed ad lib. a diet containing varying levels of magnesium (12, 24, 96 and 192 mg.%) for 28 days. Some from each group were then sacrificed. For the next 32 days all rats, control and exptl., ate a diet containing 24 mg.% Mg. The experimental diet contained, in addition, cholesterol (1%) and cholic acid (0.3%). All animals were fed 15 gm daily. The total weight gains of the 24, 96 and 192 mg.% Mg-primed groups were approximately equal but the heart-valve aorta lipidosis scores were 7, 1 and 1. The serum cholesterols, 300 - 400 mg.%, and liver cholesterols did not explain these differences. The 12 mg.% Mg-primed group gained far less, had a lipidosis score of 3 and serum cholesterol of 370 mg.%. Control groups; non-lipidotic and normocholesterolemic. Expt. II. Rates of growth were controlled by feeding, in priming and exptl. periods, varying amounts of diet containing 12 - 192 mg.% Mg.

Conclusion: High Mg prefeeding protected against cardiovascular lipidosis but this was affected by weight gains in priming and cholesterol-feeding periods.

12 *Pathology*

ELECTRON MICROSCOPY OF HUMAN ATHEROSCLEROTIC LESIONS. J.C. Geer*, H.C.McGill, Jr., J.P.Strong, and R.L.Holman. Dept. Path., L.S.U. School of Medicine, New Orleans, La.

Good preservation of much of the fine structure of coronary arteries and aortas has been obtained utilizing tissue 1 to 2 hours post mortem fixed 12 to 24 hours in formalin. Selected lesions from the formalin fixed tissue are cut into small blocks and post-fixed in osmium tetroxide. In fatty streaks the fat is intracellular, usually in smooth muscle, and involves none of the recognizable organelles of the cytoplasm. In younger individuals most of the fat has a finely reticulated structure similar to that of brown fat. In older persons there is less reticulated fat and more dense homogeneous fat droplets. Fibrous plaques are similar to fatty streaks, differing only in the proportions of the various elements--there being more connective tissue in the fibrous plaque. No fibrin or capillaries were identified in either fatty streaks or fibrous plaques. No lipid was found,other than cholesterol crystals, that could be definitely identified as extracellular. The fact that fat is intracellular, especially in smooth muscle, suggests that the accumulation of fat in atherosclerotic lesions is the result of metabolic processes in the cells of the arterial wall.

13 *Nutrition*

DECOMPENSATION OF STEROL METABOLISM IN MONKEYS. George V. Mann. Vanderbilt Univ. School of Med., Nashville, Tenn.

Restriction of protein intake induces hypercholesteremia in chickens, rats, rabbits, dogs and probably in human beings. The evidence suggests that the effective restriction is one of sulfur amino acid metabolism by limitation of cysteine precursors. A useful regimen for studying sterol metabolism and atherogenesis consists of a low sulfur diet with 1% cholesterol. This level of cholesterol is optimal for studying compensating mechanisms. Several primate species including Cebus, Lagothrix, Rhesus and Chimpanzee will develop hypercholesteremia with this regimen. This effect is blocked or cured with l-cysteine, l-cystine, dl-methionine, thiazolidine-4-carboxylic acid, cysteamine or taurine. Cysteic acid and cystamine are inactive. Both coenzyme A and pantothine parenterally are active. These compounds are approximately equivalent when expressed in relation to sulfur content. Pantothenic acid deficiency does not cause hypercholesteremia. The data suggest that sulfur and sterol metabolism are interrelated. (Supported by the A. H. A. and the N. I. H.)

14 *Nutrition*

THE EFFECT OF CALCIUM ON SERUM LIPIDS IN RABBITS J.M. Iacono, J.F. Mueller, and C.B. Ammerman (intr. by C.J. Koehn) Univ. of Cincinnati Coll. of Med., Cincinnati, Ohio.

The object of these experiments was to study the effect of undernutrition on the rate of clearing of injected fat emulsion (Lipomul) from the bloodstream of rabbits, since studies in humans have suggested that "sick" people clear infused fat emulsions at a faster rate than healthy subjects. It was shown that although suboptimal feeding for a period of 10 days had no effect on the clearing rate of injected fat when compared to ad libitum fed animals, starved animals showed a definite decrease in the rate of clearing. In addition, the fasted animals showed sharp increases in serum cholesterol and total fatty acids, with variable changes in phospholipids. In experiments designed to determine which macronutrient(s) or micronutrient(s) were effective in maintaining normal serum lipids in the ad libitum fed animals, it was found that glucose or the mineral mixture were equally effective. In subsequent screening studies of the minerals, calcium appears to be the only active substance. Serum calcium levels show consistent decreases in acute starvation which can be overcome by force feeding of calcium carbonate. The results of the experiments reported suggest a metabolic interrelationship between calcium and body lipids. Supported by a grant from the SG US Army No. DA-49-007-MD-800.

15 *Nutrition*

EFFECTS OF β-DIETHYLAMINOETHYL DIPHENYL PROPYLACETATE HYDROCHLORIDE (SKF NO. 525-A) ON BIOSYNTHESIS AND DISTRIBUTION OF CHOLESTEROL IN THE RAT. Edward G. Rice* and Samuel M. Greenberg. Res. & Dev. Div., Smith Kline & French Labs., Philadelphia, Pa.

Reports from this laboratory have shown that SKF No. 525-A reduced the serum cholesterol of dogs, mice and rats. Prolonged administration of the drug in these species caused fatty infiltration of the liver. The effect of SKF No. 525-A on cholesterol synthesis and distribution was studied in vivo in mature rats for 48 hours after injection of a single dose of mevalonic acid-2-C^{14} (MVA). Three groups were used: 1) control, 2) treated with SKF No. 525-A both before and after MVA, and 3) treated with SKF No. 525-A only after MVA. Seven days' treatment with SKF No. 525-A reduced serum cholesterol and increased liver lipids. Liver radioactivity in the drug-pretreated animals differed from the controls in that more activity accumulated in the non-saponifiable fraction, only part of which was digitonide precipitable. Serum radioactivity suggests that the drug may have interfered with liver-serum transport of cholesterol. When SKF No. 525-A was administered only after MVA dosing, liver retention of radioactivity also occurred, but much less than in the pretreated animals. The depressing effect of the drug on serum radioactivity was also less than in the pretreated animals.

007. ATHEROSCLEROSIS–Experimental

1 *Physiology*

Modification of Hypercholesterolemia and Hepatic Cholesterosis N. R. Di Luzio, Dept. Physiol., Univ. Tenn., Memphis, Tenn.

Earlier studies indicated that Kupffer cells are not primarily involved in the uptake of plasma cholesterol but may participate in its biotransformation or excretion. Increased tolerance to ingested cholesterol as reflected in lower tissue cholesterol levels should therefore be manifested during reticulo-endothelial (RE) hyperfunction. The RES was stimulated by the intravenous injection of zymosan in normal rats and in rats maintained on high cholesterol-atherogenic diets. Marked hepato-splenomegaly occurred. No alterations in various tissue lipids were observed in normal rats treated with zymosan. In rats maintained on an atherogenic diet for 14 days, the ester cholesterol of liver was 70 times and plasma 21 times greater than the control group. Zymosan treatment induced a decrease in ester cholesterol of liver and plasma of 70% and 50% respectively. Pair-feeding a 1% cholesterol, 0.5% sodium cholate diet for 5 days produced an elevation in liver ester cholesterol 58 times greater than the control value. A 90% decrease was noted in the zymosan treated group. It appears that a major determinant to elevated tissue cholesterol levels during excessive cholesterol intake is the functional state of the RES and specifically the number and activity of Kupffer cells. (Supported by a grant from AEC & PHS-H2320)

XUM

2 **Physiology**

A QUANTITATIVE ANALYSIS OF THE DEVELOPMENT OF EXPERIMENTAL CANINE ATHEROSCLEROSIS. Leroy E. Duncan, Jr., Katherin Buck, and Almorris Lynch (intr. by Bert R. Boone) National Heart Institute, Bethesda, Maryland.

The cholesterol increments at sites along the inner layer of the aorta of each of 21 dogs fed thiouracil and cholesterol were computed by subtracting from the values for those sites the corresponding mean values obtained from 20 normal dogs. After about 30 days on the regimen, the increments were greatest in the ascending aorta and progressively less down the length of the aorta. However, at about 150 days the gradient was gone, and the increment was greatest in the abdominal aorta. The early data suggest cholesterol enters aortic wall with a gradient of rates. The latter data suggest cholesterol leaves abdominal aorta slower than it leaves thoracic aorta. Thus atherosclerosis afflicts abdominal aorta especially, not because cholesterol enters that site unusually rapidly, but because it leaves there unusually slowly. Albumin enters aortic wall with a gradient of rates similar to that shown here for cholesterol. The theory that, in atherosclerosis, lipoproteins containing cholesterol enter aortic wall by the same mechanism as albumin satisfactorily explains the similarity of gradients, for two proteins entering by the same mechanism should show similar gradients. Thus these data support the filtration theory of atherosclerosis.

3 **Physiology**

THE INFLUENCE OF EXERCISE ON EXPERIMENTAL ATHEROSCLEROSIS OF HENS FED AN "ATHEROGENIC" DIET. Harry Y. C. Wong, Beatrice Liu, Rose Shim and Frank B. Johnson[1] (intr. by S. N. Stein) Dept. of Physiology, Howard University School of Medicine and Armed Forces Institute of Pathology[1], Washington, D. C. The present study was undertaken to determine the effects of exercise on blood cholesterol, aortic and coronary atherosclerosis of 45 week-old, egg-laying hens fed an "atherogenic" diet of 2% cholesterol and 5% cottonseed oil added to the mash. After 12 weeks on this regimen the following observations were noted: 1) cholesterol fed hens which were not exercised had the highest incidence of aortic atherosclerosis and a marked elevation of blood cholesterol when compared to the controls on plain mash; and 2) birds which were on an "atherogenic" diet and exercised did not show a decrease in aortic atherosclerosis or any changes in the blood cholesterol level when compared to birds on a cholesterol diet only. This is contrary to previous reports by other investigators as well as from our laboratory that exercise resulted in a significant reduction in blood cholesterol with subsequent changes in aortic and coronary arteries. Photomicrographs of the coronary arteries will be presented. (Supported in part by the National Heart Institute #H-2420, Washington and American Heart Associations)

4 **Biochemistry**

3,5 DIIODOTHYROACETIC ACID AND THE CONTROL OF TISSUE CHOLESTEROL AND LIPID CONCENTRATIONS. W.R. Ruegamer and F. R. Silverman* State U. of N.Y., Coll. of Med. and V.A. Hosp., Syracuse.

Of 33 thyroxine analogues tested, 3,5 Diiodothyroacetic acid (Diac) appeared to exert the greatest preferential effect on tissue cholesterol and lipid concentrations without affecting oxygen consumption and growth in the hypercholesterolemic rat. The optimal concentration of Diac required for both the prevention and reversal of hypercholesterolemia was 5 mg. % in the diet. Large oral doses of thyroxine and Diac produced a 50% increase in oxygen consumption as contrasted to 100-200% obtained by others using a chow diet. The high fat and 1% cholesterol content of our diet was responsible for this "sparing" action. A vitamin K deficiency and subsequent fatal hemorrhage was produced by feeding the experimental diet containing 5 mg. % Diac for 10 weeks. Menadione supplementation in the diet restored prothrombin times to control values, and no further injurious effects were encountered after 5 months' supplementation with Diac. As to the mechanism of action, dietary C-14 cholesterol absorption was unaffected by Diac feeding. However, Diac greatly enhanced the rate with which cholesterol and lipid were mobilized from liver depots. For the prevention and reversal of hypercholesterolemia in the rat, a thyroxine-like molecule with a side chain of at least 2 carbons is needed. The phenol group is not important, but at least 1 iodine in position 3 is required.

5 **Biochemistry**

THERAPEUTIC STUDIES ON SPONTANEOUS ATHEROSCLEROSIS IN PIGEONS. T.B. Clarkson and H.B. Lofland (Intr. by M.A. Swanson). Bowman Gray School of Medicine, Winston-Salem, N.C.

Five dietary or therapeutic regimes were studied for their ability to cause regression of atherosclerotic lesions occurring spontaneously in aortas of White Carneau pigeons(Clarkson et al., AMA Arch.Path. 68:143, 1959). Groups of 17 seven-year-old birds were maintained for one year on diets containing, respectively, safflower oil, 10%, Saff-Improved(Abbott), 10%, Crisco, 10%, and no supplement(controls); or vanadyl sulfate, 0.04%, or diethanolamine, 0.04%, in drinking water. A randomly-selected sample of birds sacrificed initially showed 100% prevalence of lesions, and an average atherosclerotic index (% of surface area of aorta involved in plaques)of 13.7. After one year on the diets, the birds receiving safflower oil and Saff-Improved showed significantly lower atherosclerotic indices(8.9 and 9.9 respectively; controls, 13.4). Significant decreases in aorta total lipids and aorta cholesterol values were also noted in the two groups of birds. Atherosclerotic indices and aorta lipid values in birds receiving vanadium, diethanolamine or Crisco resembled those of the controls. Gross and microscopic observations on the atherosclerotic lesions will be summarized. (Aided by grants from the U.S.P.H.S., Am. Heart Assoc., and Abbott Laboratories)

6 **Pathology**

FRACTIONATION OF SERUM LIPIDS IN RATS FED "INFARCT-PRODUCING DIET". Minoru Suzuki*and Robert M. O'Neal. Dept. Pathology, Washington U. Sch. Med., St. Louis, Mo.

If Wistar albino rats are fed 40% saturated fat supplemented with propylthiouracil, cholesterol and sodium cholate, arterial thrombi with infarcts of heart or kidneys develop in a considerable percentage of the animals (Hartroft et al. 1957). Serum lipid fractions of rats fed the basal "infarct-producing diet" were determined during the terminal 9 weeks of the 5-month experimental period. Between 5 rats that developed infarcts and 8 rats that did not, all 13 fed the basal infarct-producing diet, average values of total cholesterol (1,979 vs. 1,766 mg%), esterified cholesterol (31.4 vs. 28.7 F.A. mEq/L), phospholipids (28.5 vs. 27.8 F.A. mEq/L), total esterified fatty acids (63.4 vs. 67.1 F.A. mEq/L), or triglycerides did not differ. Rats fed the same diet with the cholesterol omitted, neither developed infarcts nor comparably high serum lipid levels. Therefore, between groups of rats fed different diets, a rough correlation between infarcts and high serum lipid levels did exist, but none of these fractions of serum lipids was useful in predicting which rats in any one group are most likely to develop infarcts. (Supported by grants from the National Heart Institute (H-1820) and from the Nutrition Foundation. Dr. Suzuki is a U.S.P.H. Trainee in Experimental Pathology (CRT-5017)).

7 **Pathology**

ABNORMAL ADRENAL FUNCTION IN ARTERIOSCLEROTIC RATS. G.W.Kittinger*,B.C.Wexler,and Benj.F.Miller. May Institute for Medical Research, Jewish Hosp. of Cinn.; Depts. of Biochem., Path., and Med., U. of Cinn.College of Medicine, Cincinnati, Ohio.

Male and female Sprague-Dawley rats fed a normal diet and bred repeatedly develop arteriosclerosis spontaneously. These animals show marked adrenal hypertrophy and thymic involution. This is especially pronounced in female breeders which exhibit gross arterial pathology. Adrenals removed from female breeders 10 to 12 months old having littered 4 to 5 times were pooled according to the degree of gross aortic pathology,e.g.,none minimal,moderate,and severe. Adrenals were incubated in vitro with a challenging dose of ACTH. Extracts of the incubation media were assayed for total α-ketolic steroids. Individual steroids were determined by quantitative paper chromatography.

Breeder adrenals showed less response to ACTH compared to virgin rats of comparable age. These findings suggest that repeatedly-bred rats are subjected to chronic overstimulation by endogenous ACTH resulting in eventual loss of capacity to meet acute demands for corticoids. Significant increases in 6-βOH-DOC, Cmpd.A, and unidentified trace steroids have been observed with increasing severity of arteriosclerosis. In some animals there is a parallel increase in DOC.
(Supported by Grant H-3874, U.S.P.H.S.)

8 **Pathology**

CHOLESTEROL RESPONSE IN RABBITS OF VARIOUS BREEDS AND HYBRIDS. Elizabeth Gaman and DeWitt Hendee Smith (intr. by Charles W. Mushett), Arteriosclerosis Laboratory, New Jersey Neuro-Psychiatric Institute, Princeton, New Jersey.

Some of the high variability of rabbits as experimental animals in atherosclerosis research might be due to the kind of rabbit being used. Preliminary tests on several breeds and some hybrids have shown interesting differences. Litters of rabbits were fed 1% cholesterol for 4 weeks, then sacrificed. Flemish had highly variable cholesterol levels and no atheroma. Chinchilla, New Zealand, Californian, English Spot, Checkered Giant, Champagne D'Argent and some of their hybrids showed varying results. In general the closely bred animals showed more consistent cholesterol levels, but varying degrees of atheroma. Hybrids showed much more uniformity in the atheroma. The commonly used New Zealand showed high variability, both within litters and between different litters, appearing to be a poor breed for this work. It is suggested that more attention to the breeding of rabbits used in atherosclerosis research can improve the consistency of results.

(Supported in part by grants from the National Heart Institute.)

9 **Pathology**

THE EFFECT OF ESTROGEN AND ESTROGEN-LIKE COMPOUNDS ON ATHEROSCLEROSIS IN CHOLESTEROL FED COCKERELS. William Peck, William Woods and Michael Radakovich (intr. by J. L. Orbison). Departments of Pathology and Surgery, University of Rochester School of Medicine, Rochester, N. Y.

The aim of this experiment was to determine the relationship between feminizing action of estrogens and their effect on atherogenesis by using estradiol and 3 estrogen analogues. Control groups consisted of animals on normal diet and normal diet plus cholesterol. All animals were sacrificed after 3 months. Estradiol produced marked feminization with increased aortic atherosclerosis and decreased coronary atherosclerosis, as compared to both control groups. Estradiol apparently influenced the metabolism of the aortic and coronary vessel walls differently. The analogues produced no feminization or statistically significant change in atherosclerosis, as compared to the estradiol group. Under these conditions no definite evidence is obtained to support the possibility that feminizing activity and the influence on atherogenesis can be dissociated in estrogen-like compounds. However, one analogue produced suggestive changes in atherogenesis though not statistically significant ones.

10 **Pathology**

A CORRELATIVE STUDY OF DIETARY, SERUM, AND TISSUE CHOLESTEROL IN RABBITS. J. M. Merrill. (intr. by G. R. Meneely.) VA Hospital, Nashville, Tennessee.

The intensity of cholesterol atherosis in rabbits has been considered to be related to both the degree and the duration of the hypercholesterolemia. To test the first portion of this hypothesis, serum cholesterol was determined at weekly intervals in six groups of rabbits fed 2% cholesterol in rabbit chow. After 8 weeks of the experimental diet, the animals were killed and the liver and the aorta tissue cholesterol determined. No significant correlation was found between any two of the following determinations: average serum cholesterol, final serum cholesterol, aortic tissue cholesterol and liver cholesterol. These results suggest that the aortic tissue cholesterol is not necessarily determined by the intensity of the hypercholesterolemia.

11 **Pathology**

THE HYPOCHOLESTEREMIC POTENCY OF OROTIC ACID IN THE PRIMATE. L. C. Fillios* and S. B. Andrus. Harvard School of Public Health and Harvard Medical School, Boston, Mass.

Certain dietary pyrimidines can influence cholesterol metabolism and the pathogenesis of atherosclerosis in the rat (Fed. Proc., 17: 436, 1958) (Am. J. Clin. Nutrition, 7: 70, 1959). More recently, it was shown that dietary orotic acid protects female rats against experimental hypercholesteremia and cardiovascular lipid deposition. Furthermore, this pyrimidine protects against the hypercholesteremia and atherogenicity induced with either uracil or thiouracil; orotic acid also elicits a protection against the thyroid hyperplasia produced by uracil (Circulation Research, 8:1, 1960). Essentially, the present report deals with the hypocholesteremic potency of orotic acid in the primate. Cebus monkeys were maintained on hypercholesteremic regimens for several months. When these diets were supplemented with either uracil and/or orotic acid the serum cholesterol changes were consistent with those in rats. Data will include measurements of other serum fractions such as phospholipids, lipoproteins, urea, and uric acid; preliminary morphologic findings will also be presented.

12 **Pathology**

THE DEVELOPMENT OF ATHEROMATOUS DISEASE IN CEBUS MONKEYS USING SATURATED AND UNSATURATED FATS. Robert W. Wissler, Laurence E. Frazier,* Randolph H. Hughes* and Richard A. Rasmussen.* Department of Pathology, University of Chicago, Chicago, Ill.

In each of 2 similar experiments 5 pairs of young adolescent Cebus monkeys were fed similar high fat rations for periods varying from 6 weeks to 50 weeks. Soya bean oil was used in the first experiment and cottonseed oil in the second. In each experiment one member of each pair of monkeys was fed a synthetic diet containing the unaltered vegetable oil while the other monkey was pair-fed the same ration in which hydrogenated oil (I#s 75 and 82) from the same batch replaced the natural oil. The monkeys were killed in pairs at varying intervals. Four monkeys receiving a low fat commercial monkey food served as controls. Histological, chemical, and isotopic tracer studies have been done on the tissues and blood of each animal. In each experiment the monkeys fed the hydrogenated fat diet developed slightly more severe elevation of blood lipids and a greater incidence of atheromatous disease of the aorta. No lesions were observed in the controls receiving the low fat stock diet and their lipids remained much lower than those of the animals receiving any of the high fat rations. Other biochemical, histological and tracer results with H^3 cholesterol as well as C^{14} acetate and mevalonic acid will be summarized.

(Supported by a grant from the Nutrition Foundation.)

13 **Pathology**

THROMBOELASTOGRAPHIC STUDIES OF FIBRINOLYSIS IN RATS FED DIETS CONTAINING BUTTER VERSUS PURE SATURATED FATTY ACIDS
R. F. Scott, W. A. Thomas and K. I. Alousi. Department of Pathology, Albany Medical College, Albany, New York.

The thromboelastograph is an electronic device that records simultaneously on a rotating film changes in the physical state of three samples of plasma from fluid state to clot to fluid state again. Thus, it is possible to compare lysis times in three samples of plasma under carefully controlled conditions. In a previous experiment by O'Neal, Tillman and Thomas in St. Louis (Circ. 20:989; 1959), it was shown that diets containing, among other ingredients 40% butter, prolonged lysis times in the plasma of rats as compared with diets containing corn oil. In the present experiment an attempt has been made (1) to confirm the previous results with butter, and (2) to find out whether the essential feature of butter is the degree of saturation by utilizing a control group in which pure saturated fatty acids were substituted for butter. Previous results with butter were confirmed. In the other portion of the experiment no change was produced by the pure saturated fatty acids but definitive conclusions are not warranted since less total food (through more saturated fatty acids) was eaten by rats fed pure acid than by those fed butter. Further experiments are in progress. (Supported by U. S. P. H. S. Grant H-4727).

XUM

14 *Nutrition*

PROTEIN-FATTY ACID INTERRELATIONSHIPS. Gladys A. Emerson and Jane B. Walker*. University of California, Los Angeles.

The effects of safflower oil and butter fat were studied in reference to growth, protein efficiency, N retention and plasma cholesterol in rats fed whole egg protein, beef protein (Rutger's) and casein (NBC). The fats were fed as 1% and 5% and the proteins as 9% of the rations. In general, egg protein promoted a growth response in young rats that was superior to that observed with the Rutger's beef protein or casein at both levels of butter fat and safflower oil intakes. The protein efficiencies followed the same pattern. Safflower oil appeared to increase protein efficiency to a greater extent than did butter fat. N retention in adult female rats was somewhat greater with casein than with beef or egg proteins. N retention was higher for the groups fed butter fat than for those given safflower oil. The total and free plasma cholesterols were highest for the 1% safflower oil and lowest for the 5% safflower oil. In general, the rats fed egg protein showed the lowest plasma cholesterols. Findings with 18% of casein and of egg protein will be reported. (Supported by a grant from the National Live Stock and Meat Board.)

15 *Nutrition*

EFFECTS OF WATER-SOLUBLE ESTERS OF SOYBEAN STEROLS ON CHOLESTEROL LEVELS IN BLOOD AND LIVER. David C. Herting and Philip L. Harris, Research Labs., Distillation Products Industries, Div. of Eastman Kodak Co., Rochester, N. Y.

Water-soluble derivatives of mixed soybean sterols were synthesized and tested for their effect on tissue levels of cholesterol in weanling male rats. The basal diet was comprised of 25% lard, 20% casein, 44% sucrose, 5% salts, 4% cellulose, and vitamins, plus 1% cholic acid and 1% cholesterol. Addition to the diet of 3.2% soy steryl-2-carbamatoglutaric acid, K salt, or the comparable derivative of stigmasterol, campesterol, or β-sitosterol (synthesized by Dr. W. J. Humphlett, E. K. Co.) reduced levels of serum and liver cholesterol as much as or more than equivalent amounts of the unesterified sterols within 4 weeks. The effect was more pronounced after 8 weeks and was observed even when the diet contained no cholesterol. In other tests, soy steryl polyethylene glycol 1000 succinate (synthesized by Dr. Max Stern, DPI) was as effective as the unesterified sterol with the diet containing cholesterol, whether or not cholic acid was added. These results contrast with those reported by other workers, showing that long-chain fatty acid esters of plant sterols are relatively inactive. Preliminary tests show that the acute oral LD_{50} for these compounds exceeds 6 g/kg. body weight.

008. ATHEROSCLEROSIS—Nutrition

1 *Physiology*

INFLUENCE OF AVOCADOS ON SERUM CHOLESTEROL. Wilson C. Grant, VAHospital, Coral Gables, Fla. and University of Miami.

Because of the influence of unsaturated seed fats on cholesterol metabolism the effect of avocados on serum cholesterol values was studied. The Fuerte avocado used contains about 20% fat of which over 85% is unsaturated and 1 1/2% is unsaponifiable. Sixteen male patients, 27 to 72 years of age were studied. Blood samples were drawn twice weekly for from 2 to 8 weeks in control period. Total, free and ester cholesterol and phospholipids were measured. The daily intake of fats and total calories were computed. In 15 patients a partial substitution of avocado fat for the regular dietary fat was made and the blood values followed for from 2 to 12 weeks. Of the 16 patients, 8 gave significant decreases in total serum cholesterol from 8.7 to 42.8% and in phospholipid of from 12.5 to 21.0%. Decrease in esters exceeded that in free cholesterol. In 2 of these patients both control and avocado feeding periods were repeated with similar results. Of the 8 patients not showing significant decreases in serum cholesterol values, 3 were diabetics and one was hypercholesterolemic. In no individual did cholesterol values rise significantly during avocado feeding. Although the average fat and calorie consumption increased during the avocado feeding period, body weights of 9 of the patients declined from 1 1/2 to 4.8%.

2 *Biochemistry*

EFFECT OF DIETARY PROTEIN AND SULFUR-CONTAINING AMINO ACIDS ON SERUM CHOLESTEROL IN THE RAT. J. C. Seidel,* N. Nath,* A. E. Harper and C. A. Elvehjem. Dept. of Biochemistry, Univ. of Wisconsin, Madison.

Changes in dietary levels of protein, sulfur-containing amino acids or choline affect the serum concentration of cholesterol in experimental animals (Portman and Stare Physiol. Rev. 39: 407, 1959). Interrelationships among these three dietary constituents have been studied. The effectiveness of a high level of casein in lowering serum cholesterol in rats fed hypercholesteremic diets containing choline (Nath et al. J. Nutrition 67: 289, 1959) could be largely accounted for by the higher methionine intake of the animals. Additions of cystine and cysteine also lowered serum cholesterol under these conditions. The magnitude of the effects of protein and sulfur-containing amino acids was influenced by the dietary level of polyunsaturated fatty acids. Serum cholesterol concentrations of rats fed a hypercholesteremic diet lacking choline were low, but increased when methionine was added to the diet. The increase was much less than that obtained upon adding choline. Therefore, methionine appears to have two opposing effects on serum cholesterol concentration of rats fed a choline-free diet: (1) a cholesterol-elevating effect attributable to lipotropic activity (2) a cholesterol-lowering effect common to sulfur-containing amino acids.

3 *Nutrition*

EFFECTS OF VEGETABLE OILS ON HEPATIC CHOLESTEROL LEVELS. Roslyn B. Alfin-Slater, Sheila Auerbach* and Rosemary L. Shull*. University of California, Los Angeles, Cal.

Experiments were undertaken to compare the effect on certain phases of cholesterol metabolism in the rat, of various vegetable oils high in essential fatty acids. In feeding experiments, when rats were given, for 12 weeks, a diet containing 1% cholesterol together with 15% of each of the following oils - cottonseed, safflower, wheat germ, or walnut- the greatest concentration of hepatic cholesterol obtained in those animals fed the cholesterol diet with either walnut oil or safflower oil; the lowest concentration of liver cholesterol was found in animals receiving wheat germ oil; cottonseed oil-fed animals had intermediate values. Other animals were fed a cholesterol-rich diet for 12 weeks, after which the cholesterol was excluded from the diet and the ability of various oils to remove cholesterol from the liver was compared. After 9 days, the liver cholesterol of animals fed cottonseed, safflower or wheat germ oils had decreased to approximately half of that of the control animals, whereas those fed the walnut oil showed no reduction in hepatic cholesterol. It would seem that the "activity" of the vegetable oil is dependent on the process under consideration. (Supported by grants from the Viobin Corp., the Diamond Walnut Growers, Inc. and the American Heart Association.)

4 *Nutrition*

THE EFFECT OF THE CARBOHYDRATES OF LEGUMINOUS SEEDS, WHEAT AND POTATOES ON SERUM CHOLESTEROL IN MAN. Joseph T. Anderson Francisco Grande and Ancel Keys. Lab. Physiol. Hygiene, U. of Minn., Minneapolis, and Hastings State Hospital, Minn.

We have reported that a diet high in fruits, vegetables and legumes produced lower serum cholesterol (chol.) in man than a comparable diet providing equal calories from sucrose and milk sugar. More recently various carbohydrate foods were fed as supplements to a basic diet for periods of 3 weeks to 24 men. In one experiment 120 g of sucrose were replaced by leguminous seeds containing the same amount of carbohydrate. Soybean protein and cellulose were added to the sucrose diet to equalize protein and fiber. Average chol. on the legume diet was 23 mg/100 ml (SE=3.7, $P<0.0001$) lower than on the sucrose diet. When the same men ate the same diets with addition of pectin in all periods the legume diet produced average chol. 24 mg/100 ml (SE=3.8, $P<0.0001$) lower than the sucrose diet. In another experiment 120 g of sucrose were replaced by an equal amount of carbohydrate 2/3 from white wheat flour and 1/3 from potatoes. Gluten was added to the sucrose diet to equalize protein. No significant chol. difference was observed. It is concluded that the carbohydrates of legumes cause lower serum chol. than sucrose.

5 *Nutrition*

THE EFFECT OF A MEDIUM CHAIN TRIGLYCERIDE ON SERUM LIPIDS IN MAN. Sami A. Hashim* and Theodore B. Van Itallie, Dept. of Medicine, St. Luke's Hospital, New York, N. Y.

A synthetic medium chain triglyceride (MCT), containing saturated fatty acids with chain lengths ranging from C_6 - C_{12} was incorporated into a formula type feeding and given as the sole source of fat to a series of 8 human subjects. The effect of MCT on serum lipids was compared with the effects of formula diets containing isocaloric amounts of corn oil and butter. Compared with corn oil, MCT induced a slight rise in serum total cholesterol. In the subjects in whom MCT was compared with butter, an appreciable increase in serum lipids occurred when butter followed MCT, and an appreciable decrease took place when MCT followed butter. It is concluded that the cholesterol elevating effect of butter probably is not due to its content of medium chain fatty acids since MCT alone lowers cholesterol levels in relation to butter. It is difficult to explain the hypercholestermic effect of butter in terms of iodine value since the iodine value of MCT is far less than that of butter. Finally, the cholesterol lowering effect of MCT cannot be explained in terms of linoleate or other polyunsaturated fatty acid content, since such acids are absent in MCT. (Supported by a grant from the Nutrition Foundation.)

6 *Nutrition*

CHARACTERISTICS OF THE LIPIDS EXTRACTED FROM THE LYMPH OF RATS FED FRESH OR HEATED CORN OIL. A. Ueno*, M. Inoue*, M. Sugai*, V. Bhalerao*, and F. A. Kummerow. Dept. of Food Technology, University of Illinois, Urbana, Ill.

One of the main intestinal lymphatics of mature rats was ligated and the other cannulated with intermedic polyethylene tubing (PE-50) under anesthesia. The animals were kept in restraining cages and fed, via stomach tube 24 hrs. after the operation, 1 ml. of fresh corn oil or corn oil which had been heated at 200°C. for 24 hrs. They were allowed free access to physiological saline solution and a diet composed of 64% glucose, 35% casein, 5% Wesson salts and all of the essential vitamins. The lymph collected in a 24 hr. period was exhaustively extracted with ethyl ether in a separatory funnel and the combined extracts freed from solvent. The carbonyl value as well as other characteristics of the residual lipid was determined. The results indicated that the carbonyl value of the lipids extracted from the lymph obtained from rats which had been fed heated corn oil was approximately ten times higher than from those fed fresh corn oil. (Supported by grant A-1671, National Institutes of Health, Dept. of Health, Education and Welfare.

009. ATHEROSCLEROSIS–Pathophysiology

1 *Physiology*

ARTERIOSCLEROSIS IN 10,000 AUTOPSIES AND THE POSSIBLE ROLE OF DIETARY PROTEIN. B. O. Barnes, 1160 So. Colo. Blvd., Denver, M. Ratzenhofer* and G. Tscherne*, Path. Inst. Univ. Graz, Austria.

A review of 10,000 autopsies during a 5-year period in a goiter region revealed that the incidence of arteriosclerosis increased from 9% at age 30 to 72% at age 60. The incidence was higher in males, especially in the coronary arteries. About 2% of the deaths before age 60 were due to myocardial infarction. Preliminary studies indicate that the average blood cholesterol may be lower in Steirmark than in America. The Austrian consumes a little less fat but considerably less protein than the American. Experimentally increasing the animal fat (eggs, butter, cream) in the diet to 60% of the total calories did not elevate the blood cholesterol in the presence of normal thyroid function. However, substituting more protein for carbohydrate elevated the cholesterol while substituting carbohydrate for protein lowered the cholesterol in spite of the high fat diet. Diet probably influences cholesterol through the thyroid.

2 *Physiology*

HYPERCHOLESTEROLEMIA: CONSIDERATIONS IN A RATIONAL APPROACH TO ITS TREATMENT. D. Berkowitz, D. Sklaroff, and W. Likoff (intr. by H. Goldberg). Albert Einstein Med. Center, and Hahnemann Med. College, Philadelphia, Pa.

During recent times, much attention has been focused on hyperlipemia as an important factor in the genesis of atherosclerosis. On the basis of this concept, the decrease of the various blood lipid fractions, notably cholesterol, has been advocated by many. More precise methods for studying fat metabolism such as an oral radioactive fat tolerance test has revealed characteristic abnormalities in coronary artery disease, which are present more consistently than is an elevated cholesterol level. Improvement in fat tolerance would therefore appear to be a rational therapeutic objective.

Accordingly, a group of hyperlipemic subjects have been treated with dietary restriction and/or various drugs which block cholesterol absorption or endogenous synthesis and the effects of these regimens on the serum lipids and fat tolerance noted over a prolonged period.

The results indicate that these therapeutic programs, however successful in reducing the blood cholesterol levels, do not improve fat tolerance. It is not likely under these circumstances, therefore, that they produce any favorable changes on the atherosclerotic process.

3 *Pathology*

FREE AMINO ACIDS AND OTHER CONSTITUENTS OF RABBITS' BLOOD AND BLOOD VESSELS. O. J. Pollak and Araceli M. F. de Engel. Dover Medical Research Center, Dover, Del.

Free amino acids, total protein, lipid and cholesterol were assayed in blood serum and in tissue extracts of ascending, thoracic and abdominal aorta, and inferior vena cava of healthy adult rabbits. To date, of the 24 known amino acids, 18 were found in tissues but only 8 in serum. The amino acid spectrum of tissue extracts did not reflect their pattern in serum. Cystine was found in serum and in all tissues. Threonine, lysine and phenylalanine were present in most tissues. Presence of other amino acids was erratic. In aortic tissue, 14 acids were identified by chromatography, and 12 in veins. A metabolic product, alpha-aminobutyric acid, was found in some veins. Amino acid patterns of the same aortic sector differed from one rabbit to another. Composition of the three aortic sectors from one and the same rabbit differed, too. More amino acids were present in the vena cava and in the abdominal aorta than in the other two aortic segments. The total protein of the vein or the abdominal aorta was 40% lower than that of the other aortic parts. The ascending aorta had 32% higher lipid content than either thoracic or abdominal aorta and 54% more lipid than vena cava. In the ascending aorta, cholesterol was 62% higher than in other aortic segments or in the vena cava. (Supported by a grant from the National Heart Institute.)

4 *Pathology*

NATURE OF CALCIUM-ELASTIN BINDING. Shiu Yeh Yu* and Herman T. Blumenthal. Jewish Hospital, St. Louis, Mo.

The percentage of total ash and of calcium in elastic fibers isolated from the media of aortas of humans of various ages have been determined. The proportion of calcium which can be removed from these preparations by chelation have also been determined. Finally, an attempt has been made to replace the calcium removed by chelation. The results show a progressively increasing percentage of ash and calcium with age, except for the oldest age group which shows a fall. Furthermore, the proportion of calcium removable by chelation diminishes with advancing age, except for the oldest age group, in which almost all of the calcium can be removed. Only a small percentage of the removable calcium can be replaced. This study indicates that elastin contains both bound and unbound calcium, the proportions of which change with advancing age. It also indicates that the elastin of patients surviving to very old ages have a different pattern of calcium binding than is encountered in patients who die at earlier ages. (Supported by NIH Grant H-2571).

010. BEHAVIOR

Pharmacology

A CONFLICT PROCEDURE FOR THE EVALUATION OF DRUGS. Irving Geller* and Joseph Seifter. Wyeth Institute for Medical Research, Philadelphia 1, Penna.

Phenobarbital, pentobarbital, meprobamate, d-amphetamine, promazine and chlorpromazine were compared for their effects upon "conflict" behavior in the rat. These compounds were administered intra-peritoneally not more frequently than once a week. The conflict behavior was established by simultaneously rewarding with liquid food and punishing with mild pain shock every lever response made in the presence of an auditory stimulus. Appropriate adjustment of the shock intensity resulted in a low but stable output of lever responses. Under phenobarbital, pentobarbital, and meprobamate lever responding increased markedly and the rats took more shocks in order to obtain the liquid food. In contrast, under promazine and d-amphetamine responding was decreased to the point where the rats took virtually no shocks at all. A preliminary investigation with chlorpromazine seems to indicate that this compound produces both effects; responding is increased or decreased during the tone depending on the dose of the compound employed.

2 *Pharmacology*

THE NONREVERSIBILITY OF THE APPETITIVE CHARACTERISTICS OF INTRACRANIAL STIMULATION. D. A. Brodie, J. L. Malis* and O.M. Moreno*. Merck Inst. for Therap. Research, West Point, Pa.

Recent reports on intracranial stimulation suggest that the same hypothalamic area may produce appetitive and aversive behavioral responses depending on the experimental situation or on the duration of the stimulus. To test this hypothesis, adult Rhesus monkeys with electrodes implanted in the vicinity of the medial forebrain bundle were trained to press a lever in order to receive brain stimulation delivered through a suitable isolation transformer. The same monkeys were next trained to lever press on a modified Sidman avoidance schedule to avoid a foot shock. Intracranial stimulation of various train lengths, up to one hour, was then substituted for the foot shock in the aversive situation. None of the animals tested avoided the intracranial stimulation, but return to foot shock reinstated avoidance behavior. The data indicate that a particular appetitive hypothalamic area, whose rewarding characteristics supported operant behavior, did not contain an aversive component regardless of the experimental situation or the stimulus duration.

3 *Pathology*

EXPERIMENTAL TURPENTINE ABSCESSES IN AGGRESSIVE AND NON-AGGRESSIVE INBRED STRAINS OF MICE. George M. Martin*, Daniel Manring* and Earl P. Benditt. Dept. of Pathology, University of Washington School of Medicine, Seattle, Washington.

Fighting response latency (method of King) and serum copper were measured in young, sexually mature male C57BL, BALB/c, C3H/HePr, C3H/HePr-bl, C57BL x BALB/c and C57BL x C3H/HePr-bl mice before and after sub-cutaneous injection of 0.1 ml turpentine. The C57BL x C3H/HePr-bl F_1 hybrids were found to be the most "aggressive" mice. Only these animals continued to fight after initiation of the turpentine abscess. In contrast, no fights were observed in 98 encounters between 14 BALB/c mice prior to injection of turpentine. The relatively aggressive C57BL strain demonstrated the greatest elevation in serum copper while the least aggressive strain (BALB/c) showed only minimal elevation of serum copper. The gross and microscopic appearances of the abscesses did not differ significantly with the possible exception of comparatively early rupture of lesions in C57BL mice. C3H/HePr-bl is a heretofore undescribed isogenic stock recently observed as a black-coated spontaneous mutant in the C3H/HePr colony maintained by R. T. Prehn. Evidence to date indicates that these animals are significantly more aggressive than the parent strain. (Supported by grants from PHS (H-3174) and Washington State Initiative 171 Funds).

011. BEHAVIOR–Disorders

1 *Biochemistry*

SERUM ELECTROLYTES IN MENTALLY RETARDED CHILDREN FED L-GLUTAMINE. Lorene L. Rogers and Wm. Austin Newton (intr. by C.G. Skinner.) Clayton Foundation Biochemical Institute, University of Texas, Austin.

Earlier studies (Rogers and Pelton, Texas Rep. Biol. Med. 15: 84, 1957) indicate that glutamine may be effective in raising IQ scores of mentally retarded children. The relationship of glutamine administration to serum sodium and potassium levels in such children has now been investigated. Sixty mentally retarded children of the familial type, residents of Austin State School, served as subjects. Fifteen children were fed 1 g. of L-glutamine daily for 5 1/2 months, 15 were given 3 g. daily, while a similar group of 30 were fed lactose placebos. Serum samples were obtained each 6 weeks throughout the period. Serum potassium levels in both glutamine groups showed a consistent and highly significant decrease. A slight but significant decrease in serum sodium levels was observed in the group fed 1 g. glutamine but not in the group fed 3 g. No statistically significant change occurred in either sodium or potassium levels in the control group. Analyses now in progress on urine samples from these subjects seem to indicate that the observed decrease in serum potassium is not due to an alteration in electrolyte balance but rather to a shift in distribution within the body tissues. Further research will be necessary to establish the exact nature of this shift.

012. BEHAVIOR–Drug Effects

1 *Physiology*

SPONTANEOUS NACL APPETITE OF PROPYLTHIOURACIL-TREATED RATS. Melvin J. Fregly. Dept. of Physiology, Univ. Florida, College of Medicine, Gainesville.

When offered choice between water and 0.15 M. NaCl solution, both male and female rats treated with the anti-thyroid drug, propylthiouracil (0.1% in diet), drink more salt solution than water. Total fluid intake also increases. Normal rats offered the same choice generally drink similar amounts of water and NaCl solution. Increases in NaCl and water intakes were also observed in hypophysectomized rats treated with propylthiouracil (PTU). Since a spontaneous NaCl appetite is known to accompany adrenalectomy in rats, the role of the adrenals in the salt appetite of PTU-treated rats was studied in the following experiment. After a 10-day control period, 5 male rats were given PTU for 2 weeks while 5 others served as controls. Water and 0.15 M. NaCl solution intakes were measured daily. PTU treatment increased spontaneous NaCl intake within 1 week after treatment began. At the end of the 2-week period, all rats were adrenalectomized and measurements were continued for 2 more weeks. Rats given PTU prior to adrenalectomy were continued on this treatment after adrenalectomy. PTU treatment combined with adrenalectomy increased salt intake above that of adrenalectomy alone. Thus, the increased NaCl intake of PTU-treated rats begins within 1 week and is independent of either pituitary or adrenal glands.

2 *Physiology*

STEROID SUPPRESSION OF LSD INDUCED BEHAVIOR CHANGES IN RATS. J. R. Bergen and G. G. Pincus. Worcester Foundation for Experimental Biology, Shrewsbury, Mass.

Winter and Flataker (Proc. Soc. Exper. Biol. & Med. 92: 285, 1956) reported that LSD produces qualitative and quantitative impairment of function in trained rats. We have used their method to test the effectiveness of various steroids to suppress this action of LSD. One mg. quantities of steroids were administered I.P. in 0.25 ml. of a vehicle containing 25% pronylene glycol, 75% saline for three days. On the fourth day the steroid was given to each rat 1 hr. before the injection of 40 γ LSD. Rats injected only with the vehicle and LSD were tested simultaneously with the steroid and LSD treated rats, the former group providing reference values for rating the effectiveness of each steroid. Significant suppression was produced by dehydroisoandrosterone, progesterone, testosterone, 15-pregnenolone, 11-desoxycorticosterone, androsterone, etiocholanolone, and Compounds A, B and S. Other naturally occurring steroids tested were not effective against LSD.

3 *Physiology*

EFFECT OF MEPROBAMATE ON CONDITIONED ANTIDIURETIC AND ELECTROLYTE RETENTION RESPONSES. S. A. Corson, E. O'Leary Corson*, R. A. Dykman*, J. E. Peters*, W. G. Reese* and L. D. Seager*. Yale Univ. School of Med. and Univ. of Ark. Med. Center.

Dogs with exteriorised ureters were placed on a stand in a sound-shielded Pavlovian type chamber equipped with one-way mirrors. Conditioned antidiuretic responses were developed in water loaded dogs by pairing auditory signals with mild electrical stimulation of a foreleg. These antidiuretic responses were accompanied by marked retention of Na, Cl and K. Similar conditioned electrolyte retention responses were developed in hydropenic dogs. The pattern of electrolyte retention was suggestive of catechol amine release rather than that of aldosterone. Administration of meprobamate (400-1200 mg/day) abolished or markedly inhibited the conditioned antidiuretic and electrolyte retention responses. Meprobamate inhibited antidiuresis even when the auditory signals were reinforced with electrical stimuli. Control experiments demonstrated that meprobamate possessed no diuretic properties. (Supported by grants from the Commonwealth Fund, the Hoyt Foundation, the Rockefeller Bros. Fund and the Foundations Fund for Research in Psychiatry.)

4 *Physiology*

THE ANATOMICAL SUBSTRATUM OF BEHAVIORAL AND CONVULSIVE PHENOMENA PRODUCED BY METHIONINE SULFOXIMINE IN THE CAT. Meyer Proler* and Peter Kellaway. Baylor Univ. Coll. of Med., Houston Texas

Parenteral administration of methionine sulfoximine produces in the cat behavioral and convulsive phenomena similar to those described in dogs fed a diet of agenized flour. Such animals are subject to convulsive seizures of myoclonic and generalized tonic-clonic type. They also have attacks of running and scrambling associated with alteration of consciousness. Episodic behavioral changes occur in which the animals appear "fearful", show licking and masticatory movements and carry out semi-purposeful acts such as searching and turning movements of eyes and head. Bilateral removal of the temporal lobes including hippocampi does not change or diminish any of these manifestations with the possible exception of some decrease in the "fear" component. Indeed, preliminary studies indicate that complete decortication (chronic animals) does not prevent the appearance of any of the other behavioral changes, decorticate animals differing from unoperated cats only in that the clonic phase of the generalized convulsions does not occur. Surface and depth recordings of the electrical concomitants of these behavioral changes and seizures have been made.

5 *Pharmacology*

EFFECT OF SALICYLATES ON TOLERANCE OF A PAINFUL STIMULUS. Bernard Weiss and Victor G. Laties (intr. by L. Lasagna) Johns Hopkins Univ. School of Medicine, Baltimore, Md.

An electric shock delivered to a rat in an experimental chamber rose in intensity every 2 seconds. Each press of a lever in the chamber reduced the intensity by one step. There were 25 steps between the minimum and maximum values of the shock current. Four drug experiments were performed. In the first, 6 rats were given saline, 2.5 mg/kg morphine sulfate, or 250 mg/kg sodium salicylate (i.p.). Median shock levels were computed for each session. Both drugs produced a rise in median shock level. The second experiment used saline and 62.5, 125, and 250 mg/kg sodium salicylate (i.p.) in four rats. The two highest doses produced higher medians than saline. Oral doses of sodium salicylate (444 mg/kg) and aspirin (500 mg/kg) were given in Experiment 3 (4 rats). Both drugs raised the median shock level but the effect of aspirin was greater. Experiment 4 used placebo, 125 and 250 mg/kg aspirin in 5 rats. Both doses produced a rise in median shock level. (Supported by grants from the Institute for the Study of Analgesic and Sedative Drugs and from the U.S. Public Health Service, NINDB (B865).

6 *Pharmacology*

EFFECTS OF CERTAIN CENTRAL DEPRESSANTS ON MICE. James E. Weaver* and T. S. Miya. Purdue University Dept. of Pharmacology, Lafayette, Indiana.

The effect of several central depressants on spontaneous motor activity (SMA) as measured in a photocell activity cage and neurologic proficiency determined by rolling roller performance (RRP) [Dunham and Miya, J. Amer. Pharm. Assoc., Sci. Ed. 46, 208 (1957)] have been observed. White mice were injected intraperitoneally with the drugs and observed at fifteen minute intervals for a two hour period simultaneously with appropriate control animals. Both SMA and RRP were severely depressed with 5 mg/Kg of chloropromazine, but the SMA returned to control levels before the end of the test while RRP did not. Equivalent doses of promazine produced depression of SMA and RRP of much lesser intensity. Marked SMA stimulation after initial depression was observed with hydroxyzine, 32 mg/Kg, after 60 minutes, but the RRP remained depressed. After the initial 15 minute period, meprobamate, 100 mg/Kg, markedly depressed SMA while RRP remained relatively normal throughout the test period. Ectylurea, 50 mg/Kg, gave essentially the same results as meprobamate. The combination of these two tests can be successfully employed to screen daytime sedatives.

7 *Pharmacology*

PERNICIOUS PREENING TEST FOR THE EVALUATION OF PSYCHOTROPHIC DRUGS. J. Gordon Wilfon *, John F. Pauls *, and Alexander Kandel. Smith Kline & French Laboratories, Philadelphia, Pa.

The application of a pilo-cohesive on mice produces a violent, unremitting tearing out of the stiff cohering strands of hair, which we have termed "pernicious preening". Compounds with proven psycholytic or analgetic properties suppress this pernicious activity. Thus, suppression of the pernicious preening might serve to unmask potential psychotrophic or analgetic agents. Groups of ten mice pretreated with a drug and painted with a pilo-cohesive substance (collodion, varnish, "Magic Marker") are placed individually in small cages (4 inches square) serially arranged. The mice are scanned for pernicious preening activity at appropriate intervals. Among the classes of compounds examined, the phenothiazine tranquilizers and potent analgetics effectively suppressed pernicious preening. Inhibitory effects have been elicited by other pharmacologic categories such as sedatives, anticonvulsants, stimulants, amine oxidase inhibitors, antihistamines and miscellaneous compounds. With these agents, abolition of pernicious preening was generally accompanied by toxic manisfestations. Comparisons among these classes of pharmacologic agents will be made. Pernicious preening activity in rats and monkeys will be discussed.

8 *Pharmacology*

CONTROLLED STUDIES ON THE PERNICIOUS PREENING TEST IN MICE. John F. Pauls*, J. Gordon Wilfon*, Alexander Kandel Smith Kline and French Laboratories, Philadelphia, Pa.

Pernicious preening is defined as a violent, unremitting tearing out of the stiff, cohering strands of hair resulting from the application of a pilo-cohesive ("Magic Marker"). Timing of actual preening periods during successive one-minute intervals indicated that the most persistent preening activity coupled with the lowest mouse-to-mouse variability occurs during a 5-minute period starting 10 minutes after the application of the pilo-cohesive. The present method involves scanning each of a group of 10 mice for presence or absence of preening activity once every 15 seconds during the 5-minute period. A total count of 20 pernicious preening episodes is thus possible. These counts make possible the use of quantal or quantitative methods for assaying the dose which abolishes pernicious preening activity or else reduces the number of episodes. The quantal procedure requires a cut-off count which can be estimated from preliminary control data. The quantitative procedure uses the counts themselves apart from any transformation germane to the statistical analysis. The choice between these two methods was based on several different analyses of the results of repeated tests on chlorpromazine. These analyses favored the quantal procedure and allowed the estimation of the precision as well as the validity of the test under a wide variety of conditions. The procedure for choosing the method of analysis, the method itself and results for various drugs will be illustrated.

XUM

9 **Pharmacology**

DRUG EFFECTS ON ELECTROCONVULSIVE INHIBITION OF CONDITIONED AVOIDANCE RESPONSE IN RATS. Elizabeth H. Jenney, Emory Univ. Atlanta, Ga.

In rats trained to perform a conditioned avoidance response (pole jump) a single electroconvulsive shock induces an inhibition of the response for intervals of 20 min to 4 hr. This study is concerned with the duration of this inhibition and its modification by drugs. A maximal tonic extensor seizure was produced in female hooded rats via ear electrodes (70 ma, 0.3 sec) every 3 to 4 days. Single doses of drugs were administered no oftener than once a week, "drug sessions" being preceded and followed by "control sessions". The animals were tested, without reinforcement, at intervals of 20, 40, 60, 90, 120, 180 and 240 min after the seizure. The duration of the inhibition was not shortened by single i.p. doses of amphetamine, 2 mg/kg, or atropine, 4 mg/kg, administered immediately after the seizure. Two doses of nialamide, 40 mg/kg, injected 14 and 2 hrs before shock were ineffective in decreasing the duration of inhibition. Chronic administration of deanol in drinking water for 2 weeks markedly shortened the duration of both behavioral post-ictal depression and inhibition of the conditioned avoidance response. Amphetamine administered in the same manner for 2 weeks showed a similar although less pronounced effect.

10 **Pharmacology**

USE OF A BENZIMIDAZOLE DERIVATIVE WITH POTENT MORPHINE-LIKE PROPERTIES ORALLY AS A PRESUMPTIVE REINFORCER IN CONDITIONING OF DRUG-SEEKING BEHAVIOR IN RATS. Abraham Wikler, Phillip C. Green,* Herbert D. Smith* and Frank T. Pescor.* NIMH Addiction Research Center, USPHS Hospital, Lexington, Kentucky.

When water-deprived, non-addicted rats (C) and rats (E) stabilized on 200 mg/kg of morphine s.c. once daily (tested 18-22 hrs abstinent) drank a 5 mcg/ml aqueous solution of 1-(Beta-diethylaminoethyl)-2-(p-ethoxybenzyl)-5-nitrobenzimidazole methane sulfonate (IG2) avidly, and exhibited morphine-like effects within 4-7 min (66-144 mcg/kg ingested), with cessation of spontaneous skin twitches (abstinence sign) in E. During the first 3 min, intragroup differences between relative amounts of IG2 and tap water consumed from either of 2 drinking tubes were not significant, regardless of side placement of IG2, indicating inability of C or E to discriminate by taste alone. Training by pairing effect of IG2 with discriminatory stimuli (DS) is now in progress. Similar C and E were trained to operate a lever for IG2 or water rewards (with DS) when water-deprived (WD) and water-satiated (WS), all four conditions randomized and balanced. On WS days, frequencies (f) of lever-pressing for IG2 were significantly higher in E than C, suggesting acquisition of preference by E, but intragroup differences for IG2 or water were not significant. Intergroup comparisons of f under continuous WS are being made at intervals after morphine-withdrawal in E to test for relapse.

11 **Pharmacology**

A BEHAVIORAL ANALYSIS OF QUALITATIVE DIFFERENCES AMONG PHENOTHIAZINES. Roger T. Kelleher,* William C. Riddle,* and Leonard Cook. Smith Kline and French Labs., Philadelphia, Pa.

Food-deprived pigeons were trained on a stimulus-producing-response procedure. During positive periods, responding on the food-producing key was reinforced; during negative periods, responses were not reinforced. Positive and negative periods alternated unpredictably in the presence of a white stimulus light. Responses on another key (stimulus-producing key) produced a 30-second stimulus light (green or red) which indicated whether positive or negative was in effect. The birds did not respond on either key when red (negative) appeared, but responded on the food-producing key when green (positive) appeared. Oral doses of promazine (6.25-25 mg/kg), chlorpromazine (5-30 mg/kg), triflorpromazine (1.25-10 mg/kg) or prochlorperazine (1.25-5 mg/kg) markedly increased responding on the stimulus-producing key. The discrimination between red and green was intact. Response rates on the food-producing key were only slightly decreased. Oral doses of trifluoperazine (0.25-2 mg/kg) did not increase stimulus-producing response rates, and higher doses decreased all responding. Trifluoperazine produced behavioral effects which were qualitatively different from those produced by promazine, chlorpromazine, triflorpromazine, or prochlorperazine.

12 **Pharmacology**

EFFECTS OF A SERIES OF PSYCHOPHARMACOLOGICAL AGENTS ON ISOLATION INDUCED ATTACK BEHAVIOR IN MICE. Leonard Cook and Edwin Weidley.* Smith Kline and French Labs., Philadelphia, Pa.

Attack behavior in mice was developed by a modification of the isolation method described by Yen et al. (1958). After isolation periods of 25 days or longer, 75-85 per cent of mice develop attack behavior. During the first five encounters after isolation two isolated mice were paired. After this conditioning period isolated mice were then tested for attack behavior by pairing with normal mice "intruders". This type of experience maintained attack behavior for at least three months. The same proportion of mice developed attack behavior, whether the walls between adjacent isolation cages were solid metal, clear plastic (adjacent mice seen), or open wire mesh (adjacent mice seen and touched). The oral ED_{50}'s (mgm/kgm) for antagonism of attack behavior in mice were determined: chlorpromazine (11.3), prochlorperazine (7.4), trifluoperazine (5.4), imiperamine (127), morphine (25.4), meprobamate (200), phenobarbital (90), trimeprazine (9.5), methiomeprazine (7.0).

13 **Pharmacology**

ASSESSMENT OF EFFECTS OF DOPA AND DOPAMINE ON THE CONDITIONED EMOTIONAL RESPONSE IN RATS. James M. Bilstad* and Wilbur M. Benson, Dept. of Pharmacology, Univ. of Minn. Medical School, Minneapolis.

Previous studies from this laboratory failed to demonstrate an effect of epinephrine and nor-epinephrine on the conditioned emotional response (CER) in rats as induced by the Skinnerian technique. Because it was shown by Weil-Malherbe et al (Science 129:1226, 1959) that these amines do not cross the blood brain barrier, it was considered possible that certain precursor substances could enter the brain in quantities sufficient to alter behavior. To assess the effects of dopa and dopamine on the CER, a modification was used of the technique described by Sieling and Benson (Fed. Proc. 18:445, 1959). CER sessions were alternated with adaptation trials. In an effort to obtain intermediate negative inflection ratios the shock intensity was varied for the individual rats during the training period. The drugs were administered in saline as subcutaneous doses of 0.1, .01, and .001 mgms. per rat. Control animals received saline. Changes occurred in the mean inflection ratios for the drug-treated rats, but these were not greater than the changes for the control rats. Because of the great intra-rat variability in the controls, questions are raised concerning the applicability of this technique to the above problem.

14 **Pharmacology**

EFFECTS OF MESCALINE AND CHLORPROMAZINE ON TWO ASPECTS OF LOCOMOTOR ACTIVITY IN RAT. Stephan L. Chorover (intr. by M.E. Jarvik.) Psychophysiological Laboratory, NYU Coll. Med., N.Y.

A modified activity wheel (15" dia.) was used to study the effects of various drugs on performance and extinction of conditional avoidance responses (CAR) in Norwegian piebald rats. Mescaline sulfate (25 and 50 mg/kg ip) and chlorpromazine hydrochloride (5 mg/kg ip) exert similar blocking effects on the CAR. In the present study, 30 Ss were employed to evaluate the possible role of locomotor deficit in drug-induced CAR disruption. Spontaneous activity of 15 Ss was observed by permitting free running in the wheel during daily test periods of 15 min. The remaining Ss were forced to run throughout the test session at three different speeds (5 min. each of 3.3, 6.6, and 10.0 rpm). Various errors were recorded for failure to follow the motor-driven rotations of the wheel. In the doses employed, both mescaline and chlorpromazine significantly reduced spontaneous activity (the latter agent doing so more markedly). At the slowest speed (3.3 rpm) neither drug affected evoked activity. At higher speeds mescaline impaired performance while chlorpromazine did not. It is concluded that mescaline-induced reduction in locomotor behavior is due largely to ataxia, while chlorpromazine appears to reduce Ss' motivation to run. (Supported, in part, by USPHS grant M-3347 to the Psychophysiological Laboratory).

013. BEHAVIOR–Drug Effects–Excitation

15 *Pharmacology*

EFFECTS OF DRUGS ON RATE OF OPERANT BEHAVIOR DURING GRADATIONS IN FEAR. Herbert Barry, III, Sylvia Wagner and Neal E. Miller (intr. by N. J. Giarman). Yale Univ., New Haven, Conn.

Hungry rats were trained to press a bar for food on a variable-interval schedule. Periods of gradually increasing tone were interpolated into the 12-min. test sessions; electric shock, intermittently presented, was correlated in intensity with the loudness of tone. Variations were studied in duration of the progressive tone (2 or 4 min.) and manner of shock presentation -- unavoidable shock to the grid floor (CER) or shock delivered only to the bar or to the portion of the floor adjacent to the bar (approach-avoidance conflict). Under each condition a similar and consistent performance was obtained during many days of testing: the rate of bar-pressing progressively decreased to a very low level as the tone and shock increased. During occasional tests for fear alone -- progressive tone without painful shocks -- performance was almost identical. The rate of bar-pressing during tests for fear was greatly increased by sodium amytal (20 mg/kg), slightly increased by chlorpromazine (2 mg/kg) and methamphetamine (1 mg/kg), variably affected by alcohol (1200 mg/kg), and unchanged by LSD (150 μg/kg). All these drugs except methamphetamine decreased performance during the non-tone portions of the session. (Supported by Research Grant MY 2949 from the U. S. Public Health Service.)

16 *Pharmacology*

DRUG EFFECTS ON THE BEHAVIOR OF SELF-STIMULATION MONKEYS. J. L. Malis*, D. A. Brodie and O. M. Moreno*. Merck Inst. for Therap. Research, West Point, Pa.

Bipolar stainless steel electrodes were implanted in the vicinity of the medial forebrain bundle of adult Rhesus monkeys. Trains of rectangular pulses (0.2 msec. pulse width, 0.2 sec. train length, 200 pulses/sec.) were delivered through a suitable isolation transformer. The monkeys were allowed to press a lever to obtain intracranial stimulation, either on a continuous reinforcement schedule (one lever press yields one brain stimulation) or on a ratio schedule (10-150 lever presses yield one brain stimulation). Dose-response curves were obtained for the drugs on both types of performance. All drugs were injected intraperitoneally. Pentobarbital (7.5-30.0 mg/kg), chlorpromazine (1.0-5.0 mg/kg) and scopolamine (0.01-0.1 mg/kg) reduced or abolished lever pressing while caffeine (25-200 mg/kg) and d-amphetamine (0.25-2.0 mg/kg) increased the rate of lever pressing. Lysergic acid diethylamide (.050-.250 mg/kg) produced erratic lever pressing behavior which was characterized by varying periods of high rates of activity. Ratio performance was more sensitive to the effects of stimulant drugs than the continuous reinforcement schedule.

17 *Pharmacology*

METABOLISM OF HALLUCINOGENIC TRYPTAMINE DERIVATIVES S. Szara, E. Hearst and F. Putney (intr. by J. Axelrod). Clinical Neuropharmacology Research Center, NIMH, Washington, D.C.

It has been reported (Szara and Axelrod, Experientia, 15:216 (1959)) that N,N-dimethyltryptamine (DMT) is excreted as N-methyltryptamine, indolacetic acid (free and conjugated), 6-hydroxydimethyltryptamine and 6-hydroxyindoleacetic acid. Recent experiments have shown that N,N-diethyltryptamine (DET), another hallucinogen undergoes the same metabolic changes both in rats and man. In addition it was found that 6-hydroxydiethyltryptamine (6-HDET) is excreted mainly as an O-glucuronide. Quantitative spectrophotometric assay shows that in rats 35-65% of the administered DET is excreted as 6-HDET-glucuronide, indicating that the major pathway for the metabolism of DET in rats is hydroxylation in the 6-position followed by conjugation to glucuronic acid. Evidence will be presented that the behavioral changes observed in trained rats are most likely due to the metabolically formed 6-HDET rather than the administered DET.

1 *Pharmacology*

COMPARATIVE ELECTROENCEPHALOGRAPHIC STIMULANT EFFECT IN MAN OF DEANOL (DEANER), CHOLINE AND AMPHETAMINE. Carl C. Pfeiffer, Leonide Goldstein*, and Elizabeth H. Jenney, Emory Univ., Ga.

The CNS stimulant effect of deanol as first reported by Pfeiffer et al (1957) is measured quantitatively in seven volunteer subjects by the changes induced in the EEG. These measurements are made possible by the continous voltage integration of a unipolar parietal lead. Deanol in a dose of 1 mg/kg given intravenously produces a significantly stimulant effect ($p < 0.001$) after a short latent period. This confirms our previous report of the stimulant effect of deanol in the unanesthetized rabbit. Choline chloride, 1 mg/kg, also produces an apparently significant stimulant effect 5 minutes after intravenous injection. Amphetamine, 0.1 mg/kg, produces a stimulant effect ($p < 0.001$) which is at no time significantly different from that of deanol. Drugs were administered in a random order and unknown insofar as the subjects were concerned. The comparisons were to a saline injection of equal volume.

2 *Pharmacology*

THE STIMULANT EFFECT OF A DEANOL CONGENER - RIKER #566. S. T. Healy*, E. H. Jenney, L. Goldstein* and C. C. Pfeiffer. Dept. of Pharmacology, Emory University, Atlanta, Ga.

Riker #566 (N-methyl, N-ethyl, 2-aminoethanol) and its salts have been tested and compared to deanol (2-dimethylaminoethanol) for stimulant action. All doses are expressed as the base. The intraperitoneal mouse LD-50's are 934 ± 95.3 mg/kg for #566 and 1020 ± 57 mg/kg for deanol when both are administered as the p-acetamidobenzoate salts. Mice given 0.03 molar #566 in their drinking water develop spontaneous convulsive seizures after 2 weeks compared with 6 to 8 weeks for mice on a similar dosage regimen of deanol. The pentylenetetrazol threshold for #566 mice is 36% of normal after 30 days of dosage compared to 46% for various groups of deanol mice after 2 to 3 months of dosage. Rats receiving 450 mg/kg per day orally in drinking water develop seizure activity within 1 week compared to 4 weeks with deanol. Quantitative EEG studies in the trained, unanesthetized rabbit show #566 to be 2 to 3 times more effective than deanol in producing an increase in electrogenesis (Drohocki's integrative method). Chronic toxicity studies indicate #566 to be well tolerated in the rat and the dog. Preliminary studies in patients indicate that #566 is more stimulant than deanol and more effective in severely depressed patients.

3 *Pharmacology*

FACTORS INFLUENCING AMPHETAMINE TOXICITY IN AGGREGATED MICE. E.A.Swinyard, J.T.Miyahara,*H.H.Wolf*and L.D.Clark.*Depts. of Pharmacology and Psychiatry, Univ. of Utah, Salt Lake City.

Aggregation increases amphetamine toxicity in mice, an effect prevented by pretreatment with certain phenothiazines and reserpine (Lasagna and McCann: Science 125:1241,1957). To explore how aggregation increases amphetamine toxicity, the central nervous system excitability of aggregated vs. isolated mice was measured by the pentylenetrazol timed i.v. infusion test (Metrazol threshold test). Isolation of CF#1 mice for 2 hours had no significant effect on Metrazol threshold, whereas aggregation of mice for 2 hours in groups of three (in a 7 x 10 x 11 cm box) decreased the threshold by 10 to 15% ($P<.05$). However, the animals rapidly adapted to the aggregated situation in that the Metrazol threshold was not decreased if the mice were aggregated for several short periods before testing. Thirty minutes after amphetamine (100 mg/kg i.p.), Metrazol threshold was decreased approximately 45% in both isolated and aggregated mice. Amphetamine was more lethal to aggregated mice (53% dead in 2 hours) than to isolated mice (no deaths in 2 hours). In contrast, amphetamine was not lethal to mice adapted to the aggregated situation. (Aided by grant B-381, N.I.H.)

014. BEHAVIOR–Drug Effects–Inhibition

/ *Pharmacology*

EFFECTS OF METHAMINODIAZEPOXIDE HCl ON "SEPTAL RATS" (Motion Picture). William Schallek and Naitan Jev.* Hoffmann-La Roche, Inc., Nutley 10, N. J.

Lesions in the septal area of the brain produce extreme irritability in rats (Brady and Nauta, J. Comp. Physiol. Psychol. 46: 339, 1953). The film shows our technique for placing lesions in this area by passage of electric current. Unoperated rats show only mild responses to annoying stimuli (prodding with stick, lifting tail with forceps, noise of air stream); rats with septal lesions show violent reactions to the same stimuli. Methaminodiazepoxide (Librium, T.M.) HCl decreases the irritability of "septal rats" without producing ataxia. Thirty minutes after intraperitoneal injection of 2.5 mg./kg., the rat shows only mild reactions to the above stimuli. It is now so gentle that it can be picked up in the bare hand.

2 *Pharmacology*

COMPARATIVE EFFECTS OF THIAXANTHENE ANALOGUES ON THE CONDITIONED RESPONSE IN THE RAT. Joe B. Nash, George A. Emerson, John A. Faust, * and Melville Sahyun.* Univ. Texas Med. Branch, Galveston and Sahyun Labs., Santa Barbara, Calif.

S.9-888, 2-Chloro-10-{3-[1-(3-hydroxypropyl)-4-piperazinyl] propyl} thiaxanthene·2HCl and several of its analogues have been found to be inhibitors of the avoidance conditioned response in the albino rat. The ED_{50} of S.9-888 for abolition of the conditioned response is 2 mg/Kg., i. p. The LD_{50} of S.9-888, in Swiss mice, is 92 ± 6 mg/Kg., i. v., 190 ± 6.5 mg/Kg., i. p., and 617 ± 39 mg/Kg., per os. Antiemetic and minimal adrenergic blocking actions in dogs have been observed after doses of 4 - 7.5 mg/Kg., i.v. Coordinated motor function, as measured in mice with the rotarod, has demonstrated marked biochemorphic differences between these analogues. S.9-888 inhibits co-ordinated movement much less than its 2-hydroxyethyl analogue, S.9-848. Cooperative clinical studies, conducted here, have shown similarly that dyskinesias produced in man by S.9-888 are minimal compared with those induced with equivalent doses of S.9-848.

3 *Pharmacology*

ANTICONVULSANT PROPERTIES OF 2-FORMYL-PHENOXYACETIC ACID TOWARDS HYDRAZIDES. C. O. Siebenmann and C. Barbara*. Connaught Med. Research Labs., University of Toronto, Ont., Canada.

2-Formyl-phenoxyacetic acid (= 2-(carboxy-methoxy)-benzaldehyde), synthesized as Compound 371 for preparing its isonicotinyl hydrazone (Zubrys and Siebenmann, Can. J. Chem., 33, 11, 1955) has a marked anti-convulsant action in mice against seizures produced by isoniazide, nicotinic acid hydrazide, semicarbazide, carbohydrazide and thiocarbohydrazide. Compound 371 has little or no effect upon convulsions produced by thiosemicarbazide, metrazole or picrotoxin. In the case of isoniazide, given by s.c. route, the i.p. dose of the Na-salt of Compound 371, required for prevention of convulsions, is only slightly larger than that calculated on the basis of the formation of the isonicotinyl hydrazone of Compound 371. Evidence of the in vivo formation of this hydrazone was obtained by its detection in the blood and urine of mice as evidenced by its maximum absorption at 340 mμ. The very low systemic toxicity of Compound 371 (LD_{50} of Na-salt for mice: p.o. 4.2 g./kg; i.p. 1.9 g./kg.) suggests its possible usefulness as an in vivo reagent for investigating the nature of certain convulsive disorders. (Supported by a grant from the Department of National Health and Welfare, Ottawa).

015. BEHAVIOR–Drug Effects–Psychoses

/ *Pharmacology*

ANTAGONISM OF BEHAVIORAL EFFECTS OF LYSERGIC ACID DIETHYLAMIDE BY PRETREATMENT WITH CHLORPROMAZINE. Oakley S. Ray* and Amedeo S. Marrazzi. Vet. Admin. Research Labs. in Neuropsychiatry, Pgh. 6, Pa.

Work in these Laboratories has shown that the central inhibitory actions (on the transcallosal system) of psychotogenic drugs can be blocked by pretreatment with tranquilizers. This study investigated the prophylactic effect of chlorpromazine on behavioral inhibition caused by lysergic acid diethylamide (LSD-25). Rats were given a water reward for lever presses in response to an auditory stimulus which was presented about every 40 seconds. LSD-25, in 100-500 μg/Kg. doses (i.p.), eliminated lever pressing for 18-50 minutes depending on dose. Chlorpromazine, 15 minutes before LSD-25, in doses too small per se (35-70 μg/Kg.) to show behavioral effects decreased the magnitude and duration of the LSD-25 effects. Doses of chlorpromazine 30-40 times the prophylactic dose when given alone caused inhibition of behavior for about an hour. There is an evident parallelism between neurophysiological and behavioral correlates of psychotogen and tranquilizer actions. A clue to the nature of the antagonism is offered by the fact that overdose of the antagonist results in an LSD-like synaptic and behavioral effect.

016. BEHAVIOR–Learning

/ *Physiology*

INDICATIONS OF INSTRUMENTAL LEARNING, "EXPLORATORY" BEHAVIOR AND "BOREDOM" IN PLANARIA. **J. B. Best and I. Rubinstein (intr. by John Z. Hearom.) Walter Reed Army Institute of Research, Washington, D.C.**

Fresh water planarians Cura foremani were run in Y-mazes in which one tunnel branch was lighted and the other darkened during a trial. Restoration of water, withdrawn at the onset of a trial, was used as the reinforcement. Results indicate learning in a number of trials comparable to those required for mammals. Deterioration of performance under further testing and its restoration and stabilization by novelty of situation suggest primordial equivalents to "exploratory drives" and "boredom." Possible artifacts from mucous trails, biases due to natural "tropisms," nonspecific physiological changes from the experiment and direct detection of the water have been ruled out by suitable controls. During learning the planarians exhibit behavior comparable to "vicarious trial and error" and "superstitious" behavior observed in higher animals, e.g. rats. Preliminary experiments suggest a "massed trial effect" similar to that observed in higher chordates. These observations indicate (1) the term "tropism" is a crudely inept preconception of planarians' demonstrable complexity of behavior; (2) many important psychological patterns may evolutionarily predate neuroanatomical structures believed to give rise to them in higher chordates.

2 *Physiology*

CARDIAC AND MOTOR CONDITIONAL REFLEX TO FOOD IN PENGUIN. W. Horsley Gantt, Wm. S. Sladen* and Maria Simonson*. Pavlovian Laboratory, Johns Hopkins University, Baltimore and V.A. Hospital, Perry Point, Md.

Previously we have demonstrated acquired cardiac adaptations to life situations, i.e. cardiac conditional reflexes (HR-CRs) in o'possum, dog, human. The penguin is of interest because of relatively simple brain and evolutionary position. Method. A smelt was dropt from an automatic feeder every 4 min., preceded by excitatory 10" tone (T256+) alternating with inhibitory (T512-) without food. Results. In 3 penguins used: cardiac and motor orienting reflex was present, could be extinguished. HR-CR was roughly parallel to motor CR. Jan.'59, first mo., control HR=132; HR-CR=140,137. 6 mos. later (June) control HR=144; HR-CR=136,125. After 6 mos.(Dec.'59) without experimentation (retention): control HR=132; HR-CR=136,138. Readings are for 5" consecutive periods. Conclusion. Penguins form motor and cardiac orienting and CRs (excitatory and inhibitory), but retention as well as HR-CRs are less pronounced, less precise than in the dog.

017. BEHAVIOR–Motivation

1 *Physiology*

SOME FACTORS MODIFYING THE EXPRESSION OF HUMAN STRENGTH. Arthur H Steinhaus & Michio Ikai* George Williams Col., Chgo. and University of Tokyo

The amount of strength a person is able to express at any moment is often far below his maximum or "physiologic limit" and is usually called his "psychologic limit." In every recording of strength or endurance involving "all-out" performance, it is really this psychologic limit that is measured. The seriousness of the error so introduced into testing programs is not generally appreciated. The present study undertakes to throw light on the nature of the psychologic barrier that stands between a man's performance and his full capacity of strength. All measurements were made on the right forearm flexors in isometric contraction using the cable tensiometer technique of Harrison Clarke. Our data show that a loud sound (starter's pistol), a shout produced by the subject himself, and suggestion under hypnosis will in this order increasingly extend maximal strength performance up to 50% in individual cases and statistically significant averages ranging from 7.4% to 26% in a group of subjects. Alcohol, adrenaline, and amphetamine sulphate also were followed by increased performances but of lesser magnitude. The authors are of the opinion that they are dealing with psychologically induced inhibitions subjected to disinhibition by psychologic and pharmacologic interventions.

018. BIOLOGICAL OXIDATION

1 *Biochemistry*

STUDIES ON RAT LIVER MONOAMINE OXIDASE. Elliott S. Harris (intr. by R. Tomarelli.) Wyeth Inst. for Med. Res., Radnor, Pa.

The pH-activity curve of rat liver mitochondrial monoamine oxidase exhibits an inflection point at pH 7.5-7.6 when measured by substrate disappearance or ammonia production. Treatment of the mitochondria with C. atrox venom [pH 7.4; 0°C; 18 hrs.] produced a 3-1/2 - fold purification, with the enzyme remaining in the insoluble residue. This preparation exhibits an optimum pH between 7.2 and 7.4. It shows decreasing stability with increasing pH, but is stabilized by the presence of substrate. A pH of 8.0 or above produces a clarification of the suspended venom residue.

The venom-treated monoamine oxidase exhibits a stoichiometry of 1 atom of oxygen consumed per mole of substrate degraded and ammonia formed. No pigment is formed with tyramine or serotonin as substrate. The reaction is unaffected by semicarbazide. In contrast to mitochondria, CN^- does not alter the stoichiometry of the reaction, but increases the initial reaction velocity by 70%.

2 *Biochemistry*

CRYSTALLINE DIHYDROOROTIC DEHYDROGENASE. Herbert C. Friedmann and Gladys Krakow (intr. by Frank A. Loewus.) University of Chicago, Chicago, Ill.

The adaptive flavoprotein dihydroorotic dehydrogenase from Zymobacterium oroticum has been crystallized as a globulin. The flavin analyzed by three independent methods: namely, direct fluorometry, paper chromatography, and apoenzyme reactivation, was found to consist of one mole each of FMN and FAD per 62,000 g of protein. Adaptation of Z. oroticum to orotate is accompanied by an increase in both FMN and FAD in the crude bacterial extracts. The increase corresponds roughly to that of enzyme formed. The enzyme contains approximately one mole of iron per flavin. The crystalline enzyme behaves towards its substrates essentially like the previously described cruder material (J. Biol. Chem., 233, 1398 [1958]). The flavin of the enzyme is completely reduced by DPNH and only partially reduced by dihydroorotate. Under anaerobic conditions, the enzyme establishes an equilibrium between DPN-DPNH and orotate-dihydroorotate. The equilibrium constant has been determined. 5-methyl orotate, in contrast to 5-fluoroorotate, is a strong inhibitor. Lipoic acid has no effect and arsenite is not inhibitory. The effects of pH and temperature on the enzyme reaction will be reported.

3 *Biochemistry*

ENZYMATIC DEHYDROGENATION OF STEROID A RING. Charles J. Sih and R. E. Bennett (introduced by J. Fried), Squibb Institute for Medical Research, New Brunswick, New Jersey.

Cell-free extracts of a Nocardia sp. have been found capable of introducing double bonds into the 1,2 and 4,5 positions of steroids possessing either the 5α- or 5β-pregnane nucleus. In the presence of a suitable electron acceptor such as phenazine methosulfate, the cell-free extracts of this organism carried out efficient conversions of pregnane - 3,20-dione, allopregnane - 3,20-dione and progesterone to $\Delta^{1,4}$-pregnadiene - 3,20-dione. The Δ^1 -dehydrogenase can utilize both one- or two-electron acceptors such as phenazine methosulfate or 2,6-dichlorophenol indophenol. Two rapid convenient assays have been devised: a) by coupling the reduced phenazine methosulfate to mammalian cytochrome c, and measuring the absorbance at 550 mμ; b) by directly measuring the reduction of 2,6-dichlorophenol indophenol at 600 mμ. The Δ^1-dehydrogenase is a steroid-induced enzyme and is associated with small particulate matter; it has been partially purified and its properties found to differ from those of the Δ^1-dehydrogenase described by Levy and Talalay (J. Biol. Chem. 234, 2014 (1959) with respect to substrate specificity, pH-optimum and dependence on electron acceptors.

4 *Biochemistry*

ENZYMATIC DECOLORIZATION OF ANTHOCYANINS. D. M. Scheiner, * J. P. Van Buren * and A. C. Wagenknecht, N. Y. S. Agric. Expt. Station, Cornell Univ., Geneva.

An oxidative enzyme system capable of decolorizing anthocyanins has been isolated from the flesh of sweet and sour cherries. A thirty-fold purification of the enzyme, on a protein N basis, has been accomplished. The reaction is inhibited by heavy metal reagents, carbonyl reagents, and ascorbic acid, but not by sulfhydryl reagents. Purified preparations exhibit little or no activity unless catechol or other 1,2-dihydroxyphenyl compounds are present in the reaction mixture. A comparison of anthocyanase and polyphenolase activities at various stages in the purification procedure indicated that the two tended to travel together. However, the ratio of the activities of the two did vary in different fractions. This may be attributed to an observed change in pH optimum of anthocyanase with increasing purification. It is believed that at least part of the oxidative reaction is enzymatically catalyzed and that 1,2-dihydroxyphenyl compounds and their o-quinones are involved in the decolorization of anthocyanins.

5 *Biochemistry*

A NEW METABOLITE OF INOSITOL SYNTHESIZED BY A MAMMALIAN ENZYME SYSTEM. Samuel H. Love (intr. by J.H. Jones) Univ. of Pennsylvania.

The possible enzymatic phosphorylation of inositol was studied with an extract of guinea pig kidneys and ATP. When such extracts were incubated with ATP and uniformly labeled inositol-C^{14}, an acidic radioactive compound was formed which was isolated by ion exchange chromatography. The compound was shown to be different from glucuronic acid, gullonic acid and their lactones by ion exchange and paper chromatography. It was also resolved from L-ascorbic acid and common sugar phosphates. The isolated radioactive compound contained no phosphorus and did not possess u. v. absorption. It was negative in the reducing test of Nelson and Somogyi as well as the $FeCl_3$-orcinol test. A faint response was obtained when paper chromatograms were developed in alkaline silver solution. The biosynthesis of this new compound from inositol requires aerobic conditions and ATP. Further characterization of the isolated compound and its metabolic relationship to known inositol metabolites will be presented. (Supported by USPHS Grant C-2228 (C5).)

6 *Biochemistry*

THE NATURE OF THE HEME BINDING IN CYTOCHROME b_5. Philipp Strittmatter (intro. by David H. Brown.) Washington Univ. Med. School, St. Louis, Mo.

The reactive groups essential for the binding of heme in calf liver microsomal cytochrome b_5 have been studied by observing the effects of group specific reagents on apocytochrome b_5 and modified preparations of this protein. Heme was quantitatively removed by acid-acetone at -70° from cytochrome b_5 and an iodinated, acetylated derivative of cytochrome b_5. One mole of the apocytochrome b_5 reacts with one mole of heme to yield a preparation that is identical with cytochrome b_5 in its spectral and catalytic properties. The iodinated, acetylated apocytochrome b_5 reacts more slowly with heme to yield a heme protein with the same absorption spectrum as cytochrome b_5. Neither sulfhydryl groups nor tyrosyl residues are involved in these reactions. Heme binding does require one specific imidazole group and may involve one or more free amino groups. The binding of heme to the apoproteins is completely inhibited by acetic anhydride and N-acetyl homocysteine thiolactone, reagents which react with amino groups. When 0.002 μ mole of the iodinated, acetylated derivative of apocytochrome b_5 is added to 0.0024 μ mole of diazotized sulfanilic acid, heme binding is inhibited 90 to 100 percent and an azoprotein is obtained with an absorption spectrum identical with that of 0.002 μ mole of mono-(p-azo-benzenesulfonic acid)-N-acetylhistidine.

7 *Biochemistry*

THIAMIC ACID, THE NON-PROTEIN COMPONENT OF α-GLYCEROPHOSPHATE DEHYDROGENASE. Jan van Eys (intr. by L. W. Cunningham) Dept. of Biochemistry, Vanderbilt Univ. School of Med., Nashville, Tenn.

Crystalline rabbit muscle α-glycerophosphate dehydrogenase contains a prosthetic group which can be isolated from the enzyme (van Eys et al., J. Biol. Chem., 234, 2308 (1959)). The spectral properties of this grouping pointed to a quaternary thiazole nature. Alkaline hydrolysis yielded aspartic acid and a polydeoxypentose. The compound could replace the thiazole moiety of thiamine in the growth of Leuconostoc mesenteroides. These and other data suggested as the most probable structure 3-(4-methyl-5-(β-hydroxyethyl)-thiazolyl)-succinic acid. This compound was synthesized (as a racemic mixture), and the synthetic material was found to be identical in all chemical tests. It was able to reactivate charcoal-treated yeast or rabbit muscle α-glycerophosphate dehydrogenase. Additional evidence was gained from the fact that thiamine deficiency in rats resulted in a significant lowering of the soluble glycerophosphate dehydrogenase. In view of the structure the trivial name thiamic acid is proposed for the prosthetic group.

8 *Biochemistry*

ELECTRON TRANSFERRING PARTICLE FROM MYCOBACTERIUM TUBERCULOSIS. Austin L. Shug* and Dexter S. Goldman. VA Hosp., Madison, Wis.

Cell-free extracts of the H37Ra strain of M. tuberculosis are prepared by grinding washed cells in a colloid mill. Intact cells, debris, and large particles are removed by centrifugation at 35,000 x G. A particle which transfers electrons from DPNH to oxygen is obtained by centrifugation at 105,000 x G for one hour. At 25° the maximum rate of DPNH oxidation is 0.04 μM/min/mg protein; this rate is 5-fold that of the crude cell-free extract. DPNH oxidation is inhibited by CO; the inhibition is reversed by O_2. Cyanide (10^{-3}M) and azide (10^{-3}M) have no effect. The composition of the particle is under investigation. A cholate extract of the particle shows reduction peaks at 595, 560, 530 and 440 mμ. Direct examination with a microspectroscope of particles at liquid air temperature shows reduction bands at about 595, 564, 560, 550, and 530 mμ, indicating the presence of cytochromes a_1, b, b_1, and c. The presence of flavin in the particles is indicated by a bleaching in the absorption spectrum in the region of 460 mμ. The particles do not catalyze the reduction of either mammalian cytochrome c or ferricyanide by DPNH; however, these activities can be demonstrated in a soluble fraction of the cell-free extract. Difference spectra indicate that only cytochrome a_1 combines with CO. It is suggested that cytochrome a_1 is the terminal oxidase.

9 *Biochemistry*

ON THE FORMATION OF SEMIQUINONE BY THE ACTION OF LACCASE. Takao Nakamura (Intr. by Dr. Harry M. Vars), Johnson Foundation, University of Pennsylvania, Philadelphia, Pa.

Laccase was found to accelerate the oxidative formation of p-benzosemiquinone (SQ) from hydroquinone (HQ) by using a Varian EPR spectrometer with 100kc. modulation. SQ was identified by its hyperfine structure. By using a flow apparatus, the kinetics of SQ were followed with a fixed field strength on the peak of the SQ spectrum. On mixing 10mM HQ with pH 7.1 buffer under the air-saturated condition, 1μM SQ was formed by autooxidation in 4 min.; but in the presence of 2μM laccase, the SQ level went up to an equilibrium value of 3μM within 30 sec. Since SQ may exist in equilibrium with Q (quinone) and HQ, the observation of SQ does not prove it to be a first product of the enzymic reaction; but the rapid increase of SQ on adding laccase in the above experiment strongly suggests that it is. Namely, on taking up 1.0μM O_2 at the early stage of the reaction, enzymic oxidation gave 1μM SQ, while 10μM Q gave 0.25 SQ at final equilibrium when it is mixed with 10mM HQ. Kinetic data show that SQ produced by enzymic oxidation decomposes with a rate which is proportional to $[SQ]^2$, which suggests that SQ, once formed, dismutates or dimerizes before it is further oxidized to Q by the enzyme. Thus, in this experimental condition, the second step of the overall oxidation reaction (from SQ to Q) is conducted mainly by a non-enzymic process.

10 *Biochemistry*

α-HYDROXY ACID OXIDASES OF HOG KIDNEY. L. Keay*, J. C. Robinson*, R. Molinari*, and I. W. Sizer, Div. Biochem. Dept. Biol., M. I. T., Cambridge, Mass.

The oxidation of a wide range of α-hydroxy acids by a crude extract of hog kidney has been studied. The distribution of the oxidases has been followed throughout a multistage purification procedure (Robinson & Sizer, Fed. Proc. 18, 123 (1959)). Two distinct L-α-hydroxy acid oxidase fractions, each inert toward D-α-hydroxy acids and amino acids, are separated by ammonium sulfate fractionation. Each enzyme has been obtained at greater than 500-fold purity by chromatography on hydroxylapatite and DEAE-cellulose. Oxidase I catalyzes oxidation of glycolate, L-α-hydroxyisocaproate, glyoxylate, and L-lactate, but not phenyllactate. Oxidase II catalyzes oxidation of a wide range of L-α-hydroxy acids (e.g. phenyllactate, p-hydroxyphenyllactate, L-α-hydroxyisocaproate) but not glycolate or glyoxylate. Each enzyme has a characteristic flavoprotein spectrum. No cofactor requirement has been demonstrated. The stoichiometry of oxidations by Oxidase I has been examined and the utilization of oxygen and the formation of hydrogen peroxide demonstrated. 2, 6-dichlorophenolindophenol can also function as a hydrogen acceptor for both enzymes. The prosthetic group of Oxidase I can be liberated by mild acid treatment, and reactivation with FMN, but not FAD, has been achieved.

11 *Biochemistry*

ANILINE HYDROXYLATION COFACTOR OF LIPID NATURE. N. H. Sloane, Mellon Institute, Pittsburgh, Pennsylvania

Two crystalline metabolites (I and II) of PABA act as cofactors in the hydroxylation of aniline by acid-fast bacteria (J. Am. Chem. Soc. 75, 635 (1953) and Fed. Proc. 18, 325 (1959)). Hydroxylation rate studies indicated that another PABA metabolite or a derivative of I or II was the complete cofactor. Addition of the lipid fraction (to resting cells) obtained by ethanol extraction of M. smegmatis cells which had metabolized PABA stimulated the hydroxylation of aniline. In the presence of the ethanol extract (H_2O suspension) the organism hydroxylated 2.4 mμmoles of aniline per mg. in 3 hours. Control cells without added lipid hydroxylated 0.4 mμmoles in this period. In addition to reversing tetracycline inhibition, the fraction also reversed the hydroxylation-inhibition caused by 5×10^{-4}M DNP. Crystalline I and II failed to reverse DNP inhibition. The optimal pH for measuring the activity of the lipid fraction was 5. The cofactor is insoluble in water but soluble in organic solvents. A concentrate purified 2000 fold has been obtained by extraction of cells with ethanol, partition into petroleum ether and chromatography on Florisil. The relations among PABA-metabolites I, II and lipid cofactor are being studied with C^{14} ring labeled PABA. The following crystalline compounds were inactive: menadione, vitamin A, α tocopherol, calciferol and coenzyme Q.

12 *Biochemistry*

ENZYMATIC UTILIZATION OF GLUCOSE BY A BASIDIOMYCETE. H. P. Maloche, Jr. (intr. by Richard W. Jackson.) Northern Regional Research Laboratory, U. S. Dept. Ag., Peoria, Ill.

In a study of the oxidation of glucose by Lactarius torminosus grown in a synthetic medium, cell-free extracts of acetone-dried preparations were used. Hexokinase, phosphohexoisomerase, and TPN-specific dehydrogenases for glucose-6-phosphate and 6-phosphogluconate were demonstrated. In addition, TPN was reduced in the presence of fructose-6-phosphate and ribose-5-phosphate, but at a much lower rate than in the presence of glucose-6-phosphate. In manometric studies of the oxidation of glucose-6-phosphate, it was found that the ratio of CO_2 production to O_2 uptake was one, and ribose-5-phosphate and fructose-6-phosphate accumulated. Further, during the incubation of ribose-5-phosphate with the cell-free extracts, the accumulation of sedoheptulose, ketopentose, and fructose was indicated. Thus it is concluded that the oxidation of glucose by Lactarius torminosus proceeded via the pentose phosphate cycle. Since phosphofructokinase and aldolase could not be demonstrated, this cycle may be the major path of glucose oxidation in this organism.

13 *Biochemistry*

ON THE REACTION MECHANISM OF DIAPHORASE (LIPOIC DEHYDROGENASE) V. Massey and Q.H. Gibson*, Univ. of Sheffield, England.

On the addition of DPNH or reduced lipoic acids to diaphorase, there is a rapid appearance of a stable spectral band with an optimum at 530 mμ. This band also appears transitorily on the addition of dithionite; it disappears either on further reduction or reoxidation. The red band thus represents the absorption of a redox intermediate, probably the flavin semiquinone. On the addition of p-chloromercuribenzoate the enzyme becomes completely bleached. It is concluded therefore that the stability of the diaphorase semiquinone is at least partly due to interaction with an enzyme sulphydryl group. The rates of formation of the semiquinone and its reoxidation under a variety of conditions have been studied by the stopped flow method (Gibson, J. Physiol. 117,49 P. 1952). The rate of its formation with DPNH is extremely rapid, equivalent to a turnover number of > 100,000. On the other hand the rate of its reoxidation by lipoic acid is comparatively slow and depends markedly on the lipoic acid concentration. Comparison of rates of the overall catalytic reaction and reoxidation of the flavin semiquinone suggest that the latter is the rate-limiting step in the enzymic reaction. This conclusion is confirmed by experiments with lipoyl glycyl glycine, where the observed rates of reoxidation of the flavin semiquinone and the overall enzymic reaction are an order of magnitude greater than with lipoic acid.

14 *Biochemistry*

OXIDATION RATES OF ACETATE BY NORMAL AND VIRAL INFECTED CHICK MEMBRANE. Wells A. Shulls* and Bert M. Tolbert. School of Pharmacy and Dept. of Chemistry, U. of Colo., Boulder, Colo.

We have developed the Radio-Warburg, a modification of the Warburg technique. A flow of 5% CO_2 in O_2 is passed thru a flask on a shaker, a drying tube, an ionization chamber (connected to an electrometer-recorder) and a CO_2 absorber. The flask contains nutrient solution, carbonic anhydrase and tissue. The $C^{14}O_2$ production is continuously measured. At pH 7 HCO_3^- turnover time is 2 minutes and is measured for each run.

Chicken eggs were incubated 14 d and de-embryonated. The chorioallantoic membrane was minced into a modified Simms solution and washed and 8-10 g wet weight placed in the flask. Acetate-1-C^{14} was injected into the flask. The $C^{14}O_2$ produced became fairly constant after 1-2 hours. After 4 hours $HC^{14}O_3^-$ was added for measurement of the bicarbonate turnover time.

PR-8 influenza virus was used to infect 15 eggs 24 h before use. The virus seed had an E.I.D.$_{50}$ of 10^{-7}. After the metabolism measurement the flask hemagglutination titer was $10^{-2.5}$ to $10^{-3.5}$. The curves were analyzed for turnover times of the several pools. The rate of oxidation of acetate to CO_2 was calculated. It was observed that virus infection decreased the turnover time of the effective acetate oxidation pool. (Supported in part by the United States Atomic Energy Commission).

15 *Biochemistry*

N-HYDROXYLATION - A NEW METABOLIC REACTION. John W. Cramer*, James A. Miller, and Elisabeth C. Miller. McArdle Mem. Lab., Univ. of Wisconsin Medical School, Madison, Wis.

N-Hydroxy-2-acetylaminofluorene (N-HO-AAF) has been isolated from the urine of rats fed the carcinogen 2-acetylaminofluorene (AAF) and identified by comparison with the synthetic compound (Cramer, Miller, and Miller, J. Biol. Chem., Mar., 1960). The excretion of this new metabolite increases as the liver damage caused by the AAF becomes more severe and by 18 weeks accounts for about 15 per cent of the ingested AAF. N-HO-AAF is excreted as a conjugate, presumably the glucuronide. To compare the fates of AAF and N-HO-AAF 8.4 μmoles per 100 gm. body weight of either compound was administered i.p. to normal rats and to rats with moderate liver damage induced by AAF. When AAF was injected 0.3 and 3 per cent of the dose was excreted as N-HO-AAF and 0.5 and 1.5 per cent as AAF by normal and AAF-pretreated rats, respectively. When N-HO-AAF was injected, 1.5 and 15 per cent of the dose was excreted as N-HO-AAF and 1 and 7 per cent as AAF by normal and AAF-pretreated rats, respectively. In all cases paper chromatograms of ether extracts of the β-glucuronidase-treated urines revealed the presence of 3-, 5-, and 7-hydroxy-AAF. The role of N-HO-AAF in the formation of the phenolic derivatives of AAF is not clear. These studies appear to furnish the first unequivocal evidence of N-hydroxylation in animals.

16 *Biochemistry*

THE NATURE OF THE PHENOMENON OF FLUORESCENCE ENHANCEMENT IN ENZYME-DPNH COMPLEXES. Harvey F. Fisher (intr. by D. H. Basinski.) Edsel B. Ford Inst. for Med. Res., Detroit, Mich.

It is now well established that addition of any of a variety of DPN-linked enzymes to a solution of DPNH causes an increase in the intensity of fluorescence of DPNH and shifts the emission spectrum to shorter wavelengths. Hypotheses previously advanced to explain this phenomenon involved highly specific enzyme-DPNH interactions leading to changes in the electron distribution or geometrical conformation of the reduced nicotinamide ring. We have found that the increase in intensity and shift to lower wavelengths of the DPNH fluorescence does not require any such highly specific interaction, but occurs in any case in which DPNH can be adsorbed on a colloidal particle, such as albumin or Dowex 1; or in which DPNH can be made into an insoluble complex, such as that formed with zinc and phosphate ions. It is well known that the fluorescence of highly flexible molecules is increased by any bond or complex formation that reduces the number of degrees of freedom of the molecule, and thereby decreases the number of deactivating collisions that compete with fluorescent emission. Therefore, the fluorescence increase and wavelength shift, caused by the addition of an enzyme to a DPNH solution, is evidence only of the formation of a complex in which the DPNH has lost some degrees of freedom.

17 *Biochemistry*

STUDIES OF OPTICAL ROTATION IN ENZYMATIC INHIBITION. David D. Ulmer* and Bert L. Vallee, Biophysics Research Laboratory of the Dept. of Med., Harvard Medical School and Peter Bent Brigham Hospital, Boston, Mass.

The denaturation of yeast alcohol dehydrogenase at alkaline pH, like that of most proteins, is manifested by more negative specific rotation, [α], and a simultaneous decrease in the dispersion constant, λc. In contrast, the formation of an enzyme-zinc-chelate complex does not result in change of λc but only in more negative [α]. Both denaturation and chelation progressively inactivate this enzyme, but by different mechanisms. The alterations in rotational properties for each process are characteristic: simultaneous change in [α] and λc is typical of variation in the secondary structure of the protein; the change in [α] alone represents formation of an enzymatically inactive chelate complex. As expected, DPN competition for the zinc site prevents inhibition through chelation and the accompanying changes in [α] to the same degree, but not the rotational changes accompanying denaturation. In this manner the contribution of the protein and of the metal chelate to the total optical rotation in protein-metal-chelate systems can be differentiated, providing an important avenue for the resolution of mechanisms of inactivation of enzymes.

18 **Biochemistry**

FREE RADICALS ASSOCIATED WITH SUCCINIC DEHYDROGENASE ACTIVITY. Thomas C. Hollocher* and Barry Commoner. Henry Shaw School of Botany and Comm. on Molec. Biol., Wash. Univ., St. Louis, Mo.

Recent studies, using the electron spin resonance technique, indicate that free radicals are associated with the redox activity of mammalian succinic dehydrogenase (SDH). The addition of succinate to aerated aqueous suspensions of particles derived from pig heart sarcosomes produces free radicals which are associated with the particles. The free radicals persist indefinitely under anaerobic conditions or under aerobic conditions in the presence of HCN or Antimycin A. The free radical concentration is diminished by malonate and abolished by heat or by the oxidative destruction of succinate. It is found that the free radical concentration is a function of temperature, heart particle concentration, fumarate/succinate mole ratio, the sum of fumarate and succinate concentrations, and malonate concentration and that an enzyme or enzymes functioning on the reducing side of the site of Antimycin A inhibition in the succinoxidase system are responsible for these free radicals. The relationships among the above variables support the hypothesis that the free radicals constitute a particular electron configuration of a complex between SDH and a compound derived from its substrates. Studies with purified SDH preparations confirm this hypothesis. (Supported by grants from Office of Naval Research and Natl. Insts. of Health.)

19 **Biochemistry**

SOLUBILIZATION AND FAD REQUIREMENT OF MITOCHONDRIAL SARCOSINE DEHYDROGENASE. Dale D. Hoskins* and Cosmo G. Mackenzie, Department of Biochemistry, University of Colorado School of Medicine, Denver, Colorado.

The mitochondrial enzyme responsible for the oxidative demethylation of sarcosine to glycine and formaldehyde has been solubilized by subjecting rat liver mitochondria, suspended in 7.5 mM potassium phosphate (pH 7.5), to sonic oscillation for 40 minutes. When the sonicate is centrifuged for 25 minutes at 100,000 x g, complete recovery of manometric activity is obtained in the supernatant fraction. However, centrifugation for 3.5 hours sediments completely the electron transfer system and leaves the sarcosine dehydrogenase in the supernatant fraction. The activity of the soluble dehydrogenase can be measured by the reduction of 2,6 dichlorophenol-indophenol. Enzyme activity varies inversely with ionic strength and is maximal at pH 7.5. The enzyme is precipitated between 40 and 80% saturation with ammonium sulfate at pH 6.2 and the activity of this fraction is increased severalfold by the addition of FAD. On the other hand, DPN, TPN, and FMN are inactive. When methyl-labeled sarcosine is employed as the substrate, the increased rate of oxidation produced by FAD is accompanied by a stoichiometric increase in the production of $C^{14}H_2O$.

20 **Biochemistry**

NATURE OF THE MITOCHONDRIAL α-GLYCEROPHOSPHATE DEHYDROGENASE. Robert L. Ringler (intr. by Edna B. Kearney). Edsel B. Ford Inst., Henry Ford Hosp., Detroit, Mich.

Previous communications from this laboratory have described the solubilization of the mitochondrial α-glycero-P dehydrogenase from pig brain following digestion with phospholipase A. The enzyme was purified by classical methods to a Q_{O_2} of 1,200-1,700 (6- to 9-fold compared with the acetone powder). The purified preparation has been resolved chromatographically on hydroxylapatite into three enzymatically active components. Prior to resolution on hydroxylapatite, the preparation contains a considerable amount of tightly bound iron (1 mole per 3×10^5 gm. protein, Q_{O_2} 1697), very little flavin (1 mole per 5×10^6 gm. protein). Its absorption spectrum shows a maximum at 412 mμ which is slightly bleached by the substrate (shift to 415 mμ) and more extensively by hydrosulfite (shift to 425 mμ). The difference spectrum (hydrosulfite or substrate) also shows a band at about 455 mμ, but it remains uncertain whether this may be ascribed to the small amount of flavin present. Following chromatography, the enzymatically active fractions contain an increased amount of iron (about 1 fold), compared with the preparation prior to chromatography, and the absorption spectrum of each fraction still shows the maximum at 412 mμ. The bearing of these observations on the mechanism of action of the enzyme will be discussed.

21 **Biochemistry**

FURTHER EXPLORATION OF THE ACTIVE CENTER OF MONOAMINE OXIDASE (MO) WITH CYCLOALKYLAMINES. E.A. Zeller and S. Sarkar*. Dept. of Biochem., Northwestern Univ. Med. School, Chicago, Illinois.

Previous studies led to the conclusion that amines and pseudoamines possessing a phenylalkyl backbone fit better into the active center of MO than related substances. - Recently cis- and trans-2-phenylcyclopropylamine were found to be the most potent inhibitors of beef liver mitochondrial MO (pI_{50} = 6.7 to 7.2; S.Sarkar et al., Helv.chim.acta, in press). To find out whether this effect is related to the MO-blocking power of certain hydrazine derivatives (J.Biol.Chem., 214, 267; 234, 389) other cycloalkylamines were prepared or obtained from various sources (S.C.Bunce, H.Green) and tested against MO. As an example the pI_{50}-values, calculated from initial rates of O_2-uptake, are given for the following compounds: cyclopropylamine (1.5), 1-phenylcyclopropylamine (5.1), cyclohexylamine (negligible). - These results indicate that only amines resembling phenylethylamine are strongly bound to the active center of MO. They also suggest a new role for the α-hydrogen, namely of participating in the formation of a covalent bond between the substrate or inhibitor and the active center; the second α-hydrogen is removed from the substrate-enzyme complex in the course of oxidative deamination. (Supported by Grant RG-5927 from the National Institutes of Health, United States Public Health Service).

22 **Biochemistry**

PURIFICATION AND PROPERTIES OF HYDROGENASE OF DESULPHOVIBRIO DESULPHURICANS. Emanuel Riklis*and David Rittenberg. Dept. of Biochemistry, College of Physicians and Surgeons, Columbia Univ. New York.

Extraction of the enzyme hydrogenase in soluble form and its partial purification by ammonium sulfate fractionation has been previously reported (Krasna, Riklis and Rittenberg, Fed. Proc. 17, 258,1958). The absorption spectrum of the enzyme fraction shows the presence of the pigments cytochrome C_3 and desulphoviridin. Chromatography on diethylaminoethylcellulose separates the hydrogenase activity from these pigments. Stable enzyme fractions are obtained by immediate precipitation of the eluate with 95% saturation ammonium sulfate and re-solution in a small volume of phosphate buffer. This purification procedure gives a 200-fold increase in specific activity. The purified enzyme catalyzes the exchange reaction between H_2 and D_2O at the rate of 3.6×10^7 μlH_2/hr/mgN. It also catalyzes the reduction of methylene blue and of benzyl viologen at the same ratio of reduction to exchange as found in the original cells. X-ray fluorescence spectroscopy reveals the presence of iron only. The purified enzyme is inhibited by carbon monoxide. Ultracentrifugal analysis shows the presence of three different proteins, the enzyme activity being found in the fraction of low molecular weight.

23 **Biochemistry**

THYROXINE ANALOGS AND YEAST METABOLISM. S. B. Barker*, W. G. Ng*, and W. Marx. Dept. of Pharmacology, Univ. of Alabama Medical Center, Birmingham, and Dept. of Biochemistry and Nutrition, School of Med., Univ. of Southern California, Los Angeles, Cal.

Resting cell suspensions of dried bakers yeast (Fleischmann's Active Dry Yeast) maintain a relatively high metabolic rate for several hours at 37°C in the presence of 1×10^{-2} M glucose, 0.025 M KH_2PO_4 and 0.05 M citric acid / K_3 citrate buffer at pH 4.7. When the yeast cells are aerated in distilled water at 25° for 12 - 16 hours, prior to determination of the oxygen consumption, the metabolic activity of the centrifuged yeast in the above mentioned phosphate-citrate-glucose solution is greatly reduced; the magnitude of this inhibition increases with the time of incubation. Addition of tetraiodothyropropionic acid (Tetraprop) to a final concentration of 2.7×10^{-5} M restores the metabolic rate nearly to the level of the unwashed cells. The formic and acetic acid analogs are ineffective, but tetraiodothyrobutyric acid is even more active. On the other hand, when the aerated yeast cells are preincubated in the phosphate-citrate-glucose medium for 1 hour at 25° prior to addition of Tetraprop, the analog causes a pronounced depression of the oxygen consumption at 37°. In the absence of Tetraprop the metabolic activity is not modified by the preincubation.

24 *Biochemistry*

THE MECHANISM OF DOUBLE BOND SATURATION IN Δ^4-3-KETOSTEROIDS. J. S. McGuire, Jr.* and G. M. Tomkins, Yale University, New Haven, Conn. and Natl. Insts. of Health, Bethesda, Md.

The mechanism of the enzymic reduction of the 4-5 double bond of 4-androstenedione by TPNH and the microsomal Δ^4-3-ketosteroid reductase (50) of rat liver has been investigated. The electrons of the double bond of the substrate are polarized by the carbonyl group at C-3 so that C-4 is relatively negative to C-5. In an ionic mechanism where TPNH donates a hydride ion, and the aqueous medium a proton, directly to the substrate, these two species of hydrogen atoms should add to carbons 5 and 4 respectively. This was tested by carrying out the reaction in tritiated water. The radioactivity of the reduction product, androstanedione, was determined both before and after enolysis in alkali. This treatment removed all the original radioactivity indicating that a proton was added only to the 4-position as predicted. Control experiments with C^{14} labeled compounds indicated that enolysis did not result in actual loss of steroid. Furthermore, if the dihydrosteroid was further reduced enzymically to the tetrahydrosteroid, the conditions of enolysis produced no loss of H^3 counts. These results are inconsistent with the involvement of an intermediate hydrogen carrier, e.g. a flavin, in which both the hydrogen atoms would be rapidly exchangeable with the aqueous medium.

25 *Biochemistry*

ISOLATION AND DEGRADATION OF AN ACETYL-ENZYME COMPLEX FORMED WITH 3-PHOSPHOGLYCERALDEHYDE DEHYDROGENASE AND p-NITROPHENYL ACETATE. J. H. Park, B. P. Meriwether*, P. Clodfelder*, and L. W. Cunningham. Physiology and Biochemistry, Vanderbilt Univ., Nashville, Tenn.

3-phosphoglyceraldehyde dehydrogenase forms an acetyl-enzyme complex with p-nitrophenyl acetate as substrate. The enzyme:acetyl ratio is 1:2.8 to 3.4 as determined (1) spectrophotometrically at 400 mμ by the initial burst of hydrolysis, (2) precipitation with 3% TCA at 0° and hydroxamate formation, and (3) radioactivity measurements using p-nitrophenyl-C^{14}-acetate. The acetyl-enzyme complex does not form below pH 6.5 and is unstable above pH 7.5. The formation of the complex is inhibited by 3-phosphoglyceraldehyde, DPN, and iodoacetic acid. These findings are consistent with the properties previously noted for the hydrolysis of p-nitrophenyl acetate catalyzed by the dehydrogenase. The C^{14}-acetyl-enzyme has been treated with pepsin for 24 hours in .04 M HCl and an acetyl-peptide obtained by paper chromatography in butanol-acetic acid-water and further purified by paper electrophoresis.

26 *Biochemistry*

ENZYMATIC REACTION KINETICS STUDIED BY MULTIPLE AUTOMATIC RECORDING OF ABSORBANCE CHANGES. W. A. Wood, Dept. of Agr. Chem., Michigan State Univ., E. Lansing, Michigan

Automatic recording of absorbance changes resulting from enzyme reactions occurring in several cuvettes simultaneously has been accomplished with (a) Beckman DU monochrometer and light source, (b) new photomultiplier unit, (c) recording potentiometer and (d) automatic cuvette positioner. The photomultiplier power supply employs anode feedback control of cathode and dynode voltages (S.R.Gilford, et al Rev. Sci. Inst. 24, 696 (1953). Thus the photomultiplier functions at constant output but varying amplification. Changes in cathode voltage as a function of absorbance were linear from absorbance 0 to 3.0. This range or as little as 0.1 absorbance change in any part of the range could be recorded at full chart width. Simultaneous recording of changes in 2 to 4 cuvettes was accomplished by a precise clock-operated cuvette changer. Sequential recording of absorbance changes in each cuvette for short intervals gave multiple records with a single pen recorder. High positioning reproducibility and electronic stability allowed use of a 1 mm pinhole, microcuvettes, and a 0.3 ml or less reaction volume. The unit has been particularly useful in determining the K_m values of reductases for reduced pyridine nucleotides where high DPH·H or TPN·H concentrations and very low velocities were employed.

27 *Biochemistry*

DPN, DPN-ANALOG AND DPNH BINDING BY RAT LIVER LACTIC DEHYDROGENASE. C. Yvonne Karnes*, E. D. Ihnen* and C. S. Vestling. Div. of Biochem., Dept. of Chem. and Chem. Engr., Univ. of Illinois, Urbana.

As part of a broad study of LDH the following four groups of DPN-analogs have been investigated in ultracentrifugal, spectrophotometric, kinetic and inhibition experiments: 1 DPN, acetylpyridine-DPN, pyridine-3-aldehyde-DPN, the corresponding deamino compounds and di-NMN; 2 ethyl nicotinate-DPN, isonicotinic hydrazide-DPN, adenosine diphosphate ribose and di-AMP; 3 AMP, ADP, AMS and IMP; 4 NMN and nicotinamide. Two moles of DPN-C^{14} are bound per mole of LDH. LDH binds one mole of ADP^{32} per mole and no nicotinamide-C^{14}. There was no inhibition of DPN-C^{14} binding by NMN, ADP, adenosine, IMP or nicotinamide or by concentrations of o-phenanthroline sufficiently high to inhibit the catalytic activity of LDH strongly. Fluorescence studies indicate that two moles of DPNH are bound tightly and two moles less tightly at neutral pH. The compounds in Group 1 all form complexes with sulfhydride ion and serve as coenzymes with LDH. The compounds in Group 2 are strong inhibitors; those in Group 3 moderate to weak; and those in Group 4 very weak. The "AMP-end" of DPN must provide a major part of the anchor for coenzyme binding. (Supported by a grant (C-1856) from the National Cancer Institute).

28 *Biochemistry*

FREE RADICAL FORMATION BY OXIDATIVE ENZYMES. Irwin Fridovich and Philip Handler. Duke University, Durham, N. C.

The aerobic oxidation of sulfite, a chain reaction initiated by free radicals, provides a sensitive detector of such radicals which has previously been employed to study the radicals generated by illuminating solutions of dyes and by milk xanthine oxidase when acting on its substrates. This technique has now been applied to a variety of enzymes acting on their substrates. DPN-linked dehydrogenases, e.g., lactic dehydrogenase, do not initiate sulfite oxidation nor do 'simple' flavin oxidases, e.g., L-amino acid oxidase, D-amino acid oxidase and glucose oxidase. Catalase is inactive whereas azide-catalase is an effective initiator. Horse radish peroxidase initiates only in the combined presence of peroxide and a peroxidizeable substrate. Calf liver xanthine oxidase and lipoxidase are effective initiators. Cytochrome oxidase acting on ferrocytochrome c is an extremely potent initiator. The latter observation strongly suggests that cytochrome oxidase generates radicals by the stepwise univalent reduction of oxygen. H_2O_2 should be an obligatory intermediate in this process. This is substantiated by the observation that cytochrome oxidase catalyzes the oxidation of ferrocytochrome c by H_2O_2 at low O_2 tension. A detailed mechanism for the univalent reduction of O_2 to water by the cytochrome oxidase system is proposed.

29 *Biochemistry*

STUDIES ON FORMATE METABOLISM OF A PARASITIC FUNGUS, (TILLETIA CONTRAVERSA). E. B. Vaisey,* Vernon H. Cheldelin and R. W. Newburgh. Science Research Institute, Oregon State College, Corvallis, Oregon.

Cell free extracts from teliospores oxidize formate largely by means of a catalase-hydrogen peroxide complex. Evidence for this is based on the fact that formate oxidation is competitively inhibited by ethanol, inhibited by cyanide, and insignificantly inhibited by carbon monoxide. Further it has not yet been possible to demonstrate oxygen uptake, or pyridine nucleotide reduction with formate as substrate.

Pathways leading to formate are complex but in acetone powders of teliospores the following sequence of events is indicated:

(1) pyruvate ⟶ oxalate ⟶ formate + CO_2

(2) acetate ⟶ glycolate ⟶ glyoxalate ⟶ formate + CO_2

In equation 1, oxalate is labeled from pyruvate-1-C^{14} but not from pyruvate-3-C^{14}. In equation 2, formate is labeled from acetate-2-C^{14}.

30 *Biochemistry*

AMINO ACID OXIDASE - CATALYZED DEHYDROGENATION OF LEUCINE. Lourdes Tuazon*, Frederick R. Duke* and David E. Metzler. Department of Chemistry, Iowa State University, Ames.

The kinetics of the amino acid oxidase - catalyzed dehydrogenation of L-leucine by methylene blue has been studied at 25° under a variety of conditions of concentration. The enzyme was used in the form of dried cottonmouth moccasin (Agkistrodon piscivorus) venom. The methylene blue concentrations were sufficiently low to avoid competition with the amino acid. Maximum velocities and inverse Michaelis constants were evaluated at a variety of pH values. Between pH 5 and 6 the maximum velocity was essentially independent of pH, and above pH 6 substrate inhibition was encountered. The inverse Michaelis constant between pH 5 and 6 varies in such a way as to suggest that the amino acid anion combines with the enzyme with the displacement of a proton. Substrate inhibition constants have been evaluated. The kinetics of L-valine and L-alanine oxidation have been compared under more limited conditions. (Supported by grants from the National Science Foundation and the National Institute of Arthritis and Metabolic Diseases.)

31 *Biochemistry*

STUDIES ON A MICROSOMAL TPNH-NEOTETRAZOLIUM DIAPHORASE. Charles H. Williams, Jr.* and Henry Kamin. VA Hosp. and Dept. of Biochem., Duke Univ. Medical Center, Durham, N. C.

A previous report from this laboratory (Biochim. Biophys. Acta 32: 568, 1959) has shown that liver TPNH - neotetrazolium (NT) diaphorase activity resides largely in "microsomes", unlike TPNH-dichlorophenolindophenol diaphorase activity which is highest in the soluble fraction. Efforts at purification of TPNH-NT diaphorase have involved two types of procedure: (1) alkaline 20% butanol extraction, dialysis, and centrifugation at 80,000xG and (II) pancreatin digestion followed by ammonium sulfate fractionation. Procedure (I) produces in low yield a red particulate preparation with 30 times the specific activity of microsomes. This preparation when reduced with hydrosulfite shows a cytochrome b_5-like spectrum, but it is not known whether this cytochrome is an integral part of the diaphorase or an accidental contaminant. Procedure (II) yields a soluble preparation 3-fold purified from microsomes. Thus far fractions with high TPNH-NT diaphorase activity also show TPN-cytochrome c reductase activity, but data on comparative activities of these fractions toward NT and cytochrome c do not as yet permit conclusions as to the identity of this diaphorase with TPN-cytochrome c reductase; the possibility is not excluded that NT diaphorase activity may reflect some other aspect of microsomal TPN metabolism. (Supported by Grant No. E-1999, USPHS.)

32 *Pharmacology*

METABOLIC FATE OF THIOBARBITAL IN MAN. Milton T. Bush. Dept. of Pharmacology, Vanderbilt Medical School, Nashville, Tenn.

Several years ago we reported (Fed. Proc. 15, 406, 1956) that after an oral dose of 200 mg. of thiobarbital, about 1.5% was excreted in the first 36 hour urine. (The 15% given in that abstract was a typographical error.) No barbital was demonstrable by the technique then available. We have now devised improved techniques (Fed. Proc. 18, 374, 1959). Diethyl ether in the presence of air readily oxidizes thiobarbital to barbital. Therefore thiobarbital is extracted from the urine quantitatively with butyl chloride, which removes relatively little barbital. Then the barbital is extracted with diethyl ether. The substances are determined in the separate extracts by appropriate spectrophotometric measurements. Pure thiobarbital was injected intravenously (2 mg./kg.) into each of three men. All three subjects showed the same excretion pattern. Approximately 2% of the dose of thiobarbital was eliminated unchanged in the first 48 hours, and this diminished rapidly to 1%, 0.5% and 0.2% for the succeeding 48-hour periods. Barbital was eliminated at a rate of 1% per 48 hours for about 8 days, then more slowly. Thus most of the thiobarbital was metabolized in some other way, but some 5 to 10% was slowly converted to barbital. (Supported in part by Research Grant B-1494 from the National Institute of Neurological Diseases and Blindness, U.S. Public Health Service.)

33 *Pharmacology*

STUDIES ON THE MECHANISM OF ACTION OF 3-AMINO-1,2,4-TRIAZOLE. T. R. Tephly*, G. J. Mannering and R. E. Parks, Jr. Dept. of Pharmacology and Toxicology, Univ. of Wis. Med. Sch., Madison.

Heim et al. (Science 122: 693, 1955) found 3-amino-1,2,4-triazole (AT) to inhibit hepatic catalase activity and Nelson (Science 126: 1241, 1957) observed antagonism of this AT effect by ethanol. In the present study, various compounds were compared for their ability to antagonize, in vivo, the minimal amount of AT (0.3 g/kg) effecting a 90% inhibition of rat hepatic catalase activity. Methanol, ethanol and acetaldehyde in I.P. doses of 0.012 moles/kg reduced this inhibition to 14%, 11%, and 57% respectively. At an I.P. dose of 0.024 moles/kg, the following inhibitions of hepatic catalase by AT were observed: methanol 3%, ethanol 7%, propanol 47%, butanol 74%, ethylene glycol 64%. In this dose range isopropyl and secondary butyl alcohols were inactive. The AT-antagonizing ability of the alcohols seems related to their substrate activity in a catalase-peroxide generating system. In vitro studies employing crystalline beef liver catalase revealed negligible inhibition by AT alone, but with addition of a purified peroxide generating system, complete inhibition occurred. Ethanol and methanol antagonize this inhibition of crystalline catalase activity. Results of kinetic studies will be presented and consideration of the mechanism of catalase inhibition by AT discussed.

34 *Pharmacology*

STUDIES ON NICOTINE METABOLISM: THE FORMATION OF γ-(3'-PYRIDYL)-γ-METHYLAMINOBUTYRIC ACID (PMABA). H.B. Hucker* and J.R. Gillette. Natl. Insts. of Health, Bethesda, Md.

An enzyme system in liver microsomes, which requires TPNH and oxygen, catalyzes oxidation of nicotine to cotinine by way of "hydroxynicotine" (Hucker et al.: J. Pharm. Exper. Therap. in press). Since cotinine accounts for only 60% of the nicotine metabolized, it seemed possible that nicotinine might also be converted to PMABA (McKennis, et al.: J. Am. Chem. Soc. 79: 6342, 1957). Possible pathways to PMABA are (1) "hydroxynicotine" → cotinine → PMABA, and (2) "hydroxynicotine" → γ-(3'-pyridyl)-γ-methylaminobutyraldehyde (PMABAld) → PMABA. To ascertain which pathway is used in vivo, we developed a method for the assay of PMABA. Little or no PMABA accumulated during oxidation of nicotine to cotinine by the 9,000 x g supernatant system of rabbit liver. Moreover, this enzyme system did not metabolize cotinine or PMABA, and did not form cotinine from PMABA. These results suggest that little PMABAld is formed, since these enzyme preparations contain both aldehyde dehydrogenase and aldehyde oxidase. Therefore, PMABA is probably formed only by pathway 1. The results also prove that PMABA is not an important precursor of cotinine. Studies in vivo support these conclusions: PMABA was formed in rabbits given cotinine or nicotine, and no cotinine was detected in urine or rabbits given PMABA.

35 *Pharmacology*

EFFECT OF CHLORPROMAZINE (CPZ) ON LEVELS OF ADENINE NUCLEOTIDES AND CREATINE PHOSPHATE (CP) OF BRAIN. N. Weiner, Dept. of Pharmacology, Harvard Med. School, Boston, Mass.

Rats were given markedly depressing doses of CPZ and subsequently killed by immersion in liquid O_2, and the brains were analyzed for ATP, ADP, AMP, and CP. The brain levels of these compounds in treated rats were not different from those in control animals. If either the control or CPZ treated animals were briefly stimulated during killing, brain levels of ATP and CP fell, and those of AMP rose. The changes were less marked in the CPZ treated animals, with the result that in this group the ATP and CP levels were higher, and the AMP levels were lower, than those of the control animals. The data suggest the rate of utilization of labile phosphate compounds is depressed in the CPZ treated animals, probably secondary to depressed brain function. The known inhibitory effects of CPZ on enzymes of oxidative metabolism and oxidative phosphorylation could not account for the pharmacological action of the drug unless inhibition only at specific sites were postulated. (Supported by USAF SAM Contract AF 41 (657)-197 and USPHS grants H-2205 and H-3674.)

019. BIOLOGICAL OXIDATION–
Electron Transport

Biochemistry

1

CHARACTERIZATION OF A NEW ELECTRON TRANSPORT COMPONENT ISOLATED FROM MYCOBACTERIUM PHLEI. W. B. Sutton. Lilly Res. Labs., Indianapolis, Indiana.

The absence of endogenous reduction of 2,6-dichlorophenol-indophenol (2,6-DPIP) by sonic extracts following dialysis has been observed. The presence of a stable, blue fluorescent component (AM2CP) in aqueous extracts of methanol precipitated material suggested a reduced nucleotide similar to DPNH. The reducing activity of crude AM2CP can be greatly increased by the addition of a partly purified reductase from Myco. phlei. Exposure of crude AM2CP to ultraviolet light of 2537 A° destroys the endogenous activity and increases the reductase activity. On standing the endogenous activity returns. Rat-liver mitachondria will reduce 2,6-DPIP when AM2CP is present, but diaphorase does not. The failure of mitachondria to reoxidize 2,6-DPIP when reduced with AM2CP, in contrast to the rapid reoxidation when DPNH or TPNH are used, suggests that AM2CP is different from DPNH or TPNH. Ferricyanide can replace 2,6-DPIP. Purified AM2CP has absorption peaks at 260 and 345 mu, and when examined by strip electrophoresis the blue fluorescent component moved in a manner similar to DPNH.

Biochemistry

2

SUCCINIC AND DPNH OXIDASES OF ASCARIS LUMBRICOIDES. Emil Kmetec* and Ernest Bueding. Department of Pharmacology, Louisiana State University, Medical School, New Orleans, La.

Ascaris succinoxidase is devoid of measurable cytochrome c and cytochrome oxidase activities and requires Mn^{++} for optimal activity (Bueding and Charms: J. Biol. Chem., 196, 615, 1952). Mn^{++} also acts as a cofactor for the oxidation of DPNH by a particulate fraction of Ascaris muscle. In the presence of Mn^{++} both succinic and DPNH oxidases are stimulated by 8-hydroxyquinoline; their activities are dependent upon O_2 tension and result in the formation of H_2O_2. Experiments using phenazine methosulfate, methylene blue and O_2 as electron acceptors suggest a minimum of three steps in electron transfer from succinate to a terminal flavoprotein oxidase. This was confirmed by the inactivation of specific reducing sites which can be assayed separately or in combination with other constituents of this system. Anaerobically, DPNH oxidation by the particulate preparation can be coupled with the reduction of fumarate to succinate. The large amounts of succinate produced anaerobically by intact Ascaris suggest that coupled fumarate reduction may be required for glycolysis by providing a source of oxidized DPN. (Supported by NIH Grant No. E-668).

Biochemistry

3

PROPERTIES OF A SOLUBLE SUCCINIC-COENZYME Q REDUCTASE ISOLATED FROM BEEF HEART MITOCHONDRIA. K. A. Doeg* and D. M. Ziegler. University of Wisconsin, Madison, Wisconsin.

The purified succinic-coenzyme Q (CoQ) reductase from beef heart mitochondria contains 4.5 mμ moles flavin (extractable with acid only after tryptic digestion), 18 mμ moles non-heme iron and 4.8 mμ moles heme (protohemen) per mg protein. The heme group can be extracted by acetone from the lipid-free protein. The dehydrogenating activities of the enzyme (expressed as μ moles succinate oxidized/min/mg protein) with several electron acceptors are as follows: CoQ-35; phenazine-28; ferricyanide-15; and indophenol-30. The enzyme reacts rapidly with CoQ only when supplemented with small quantities of a mitochondrial lipid extract and a non-ionic detergent. The CoQ reductase activity of the enzyme is irreversibly destroyed by extracting the heme with acetone; however, the resulting heme-free flavoprotein can still catalyze the succinate-ferricyanide or succinate-phenazine reactions. The flavin, but not the heme component of the CoQ-reductase, is reduced by succinate. The heme group does not participate as an electron carrier in the reduction of CoQ, but it may be required to preserve some structural arrangement within the enzyme necessary for CoQ reduction.

Biochemistry

4

DPNH-CYTOCHROME c REDUCTASE. Y. Hatefi, A. G. Haavik, P. Jurtshuk and B. Bovee (intr. by J. D. Ferry.) Univ. of Wis., Madison, Wisconsin.

A particulate DPNH-cytochrome c reductase has been isolated from beef heart mitochondria by means of deoxycholate-KCl solubilization of the proteins followed by ammonium acetate fractionation. The preparation contains DPNH flavoprotein, coenzyme Q, and cytochromes b and c_1. It catalyzes the reduction by DPNH of 22-25 μmoles of cytochrome c or 60-70 μmoles of ferricyanide per mg. of protein per min. at 38°. With succinate, these activities are usually less than 0.2. DPNH-cytochrome c reductase activity is completely inhibited by antimycin A, SN-5949 and Amytal. TPNH (± DPN) is not oxidized by the enzyme. When the enzyme system is titrated with DPNH at 0°, reduced electron carriers appear in the following order: cytochrome c_1, cytochrome b, flavoprotein. The bound coenzyme Q is also easily reduced at 0°. When cytochrome c plus cytochrome oxidase are added to the reduced enzyme, the order of reoxidation of components is exactly the reverse of reduction. Antimycin A inhibits the reoxidation of cytochrome b and 2,3-dimethoxy-5-bromo-6-methyl benzoquinone (5-bromo-coenzyme Q_0) abolishes the antimycin A effect by acting as electron carrier between cytochromes b and c_1. Mitochondrial lipides stimulate cytochrome c reductase activity of the enzyme. The preparations are quite stable to aging at -20°.

Biochemistry

5

SULFITE ACTIVATED OXIDATION OF DPNH BY PEROXIDASE. S. J. Klebanoff. The Rockefeller Institute, New York, N.Y.

DPNH is oxidized by peroxidase in the presence of Mn^{++}, oxygen and certain phenolic compounds (Akazawa and Conn, J. Biol. Chem. 232, 403, 1958). Among the phenolic compounds which are active are thyroxine and estradiol (Klebanoff, J. Biol. Chem. 234, 2480, 1959; Williams-Ashman, Cassman and Klavins, Nature 184, 427, 1959). The Mn^{++} dependent aerobic oxidation of DPNH by horseradish peroxidase occurs at a rapid rate in the absence of a phenolic compound if sodium sulfite (or bisulfite) is added to the reaction mixture. An effect of sulfite is observed with phosphate, cacodylate and to a lesser extent arsenate buffer under conditions in which little or no effect is observed with tris, glycylglycine or imidazole buffer at pH 7. Horseradish peroxidase can be replaced in this system by myeloperoxidase, hemoglobin or myoglobin. The sulfite activated oxidation of DPNH is inhibited by thiosulfate, hydrogen peroxide, cupric ions, ascorbic acid, glutathione (reduced or oxidized) and epinephrine. Thyroxine and estradiol stimulate the Mn^{++} dependent aerobic oxidation of DPNH by peroxidase in the presence of sulfite under conditions in which little or no effect is observed in the absence of sulfite. (Supported in part by research grant A-2772 from the National Institutes of Health)

Biochemistry

6

RAPID IDENTIFICATION OF VITAMIN K HOMOLOGUES BY INFRARED SPECTROSCOPY. Hans Noll. Dept. of Microbiol. Univ. of Pittsburgh Sch. of Medicine, Pittsburgh, Pa.

Information on the occurrence of K-vitamins of precisely defined structure is scarce, because of deficiencies in the available analytical methods. UV-spectroscopy and paper-chromatography fail to distinguish between homologues of the K_1 and K_2-series of similar mol. wgt. Using pure reference compounds, K1(10,15,...30) and K2(5,10,...50) synthesized by Isler et al. /Helv. Chim. Acta 41,786(1958)/ an IR-spectroscopic method capable of precise identification of any vitamin K homologue was developed. Absorption bands near 1100 and 840 cm^{-1}, present in K_2- but absent in K_1-spectra, permit rapid qualitative differentiation between the two homologous series. The length of the sidechain is determined by absorbancy measurements in CCl_4 solution. A linear relationship is observed between the number of carbon atoms in the sidechain and the ratio of the main alkyl stretching and bending bands to the absorbancies of the characteristic naphthoquinone bands. A simple slit width calibration procedure with commercial K1(20)(F. Hoffmann-LaRoche Co.) as standard yields absorbancy ratios which are characteristic for each homologue and independent of substance concentration, cell thickness and instrument constants. The scope of the method and its application in identifying K2(45) from M. tuberculosis are discussed.

7 *Biochemistry*

THE ROLE OF "SOLUBLE LIPID" PREPARATIONS IN SUB-MITOCHONDRIAL ENZYME SYSTEMS. Sidney Fleischer and Herman Klouwen (intr. by F. Skoog.) University of Wisconsin, Madison, Wisconsin.

A "soluble lipoprotein"(Q-L.P.) which contains CoQ and 4% protein has been described by Basford and Green (BBA 33 185, 1959). This Q-L.P. is highly effective in enhancing enzymic activity in various sub-mitochondrial preparations; e.g., cytochrome c oxidase (soluble),(Griffiths et al.Fed. Proc., 1960). Q-L.P. gives 2-fold stimulation; aqueous-acetone treated mitochondria (AATM) which are practically depleted of lipid (Fleischer and Lester, in preparation) have an absolute requirement for Q-L.P. for succinoxidase activity. Total mitochondrial lipides as well as purified lipid fractions (courtesy of Dr. R. Bomstein) have been "solubilized" by treatment with Butanol + cholate and dialysis in a rocker-dialyzer for 7 days, vs. tris-EDTA (0.02-0.001 M; pH 8.0). The factors involved in "solubilization" of lipids will be discussed. Organization of the lipid rather than the 4% protein in the Q-L.P. seems to be responsible for solubility. "Solubilized" total mitochondrial lipid can completely replace the Q-L.P. in the enzyme systems mentioned. Where studied (AATM), most of the succinic-cytochrome c reductase activity is restored with phosphatidylinositol or certain combined lipid fractions, when supplemented with CoQ. In their "solubilized" form these lipids as well as those of Q-L.P. are accessible to the enzyme system.

8 *Biochemistry*

ELECTRON TRANSMITTER SYSTEM IN ADIPOSE TISSUE. Cliffe D. Joel* and Eric G. Ball. Dept. of Biological Chemistry, Harvard Medical School, Boston, Mass.

In this study it has been found that brown adipose tissue (BAT) of male rats contains high concentrations of cytochromes and ubiquinone (coenzyme Q). The presence of the cytochrome system is readily revealed by direct examination of the intact tissue with a hand spectroscope and appears to account for the characteristic color of BAT. A washed brown particulate fraction obtained from BAT, when examined in either buffered deoxycholate or concentrated sucrose solution with the Beckman spectrophotometer, revealed an abundance of cytochromes a + a_3, b and c. Estimations of the content of cytochrome c in BAT from such spectral studies have yielded values in agreement with those obtained by its chromatographic isolation. Values comparable to those reported for rat heart muscle, approximately 2 mg. per gm. of fat-free dry weight, have been obtained. The unsaponifiable lipides of the isolated BAT particles and of the nearly colorless fat layer which separated during the preparation of the particles have been analyzed for ubiquinone. Preliminary results, based on the spectral change due to reduction by borohydride, indicate that the molar concentration of this compound in the particles is ten times that of cytochrome c and that the amount of ubiquinone in the fat layer is about equal to that of the ubiquinone in the particles.

9 *Biochemistry*

A PARTICLE WITH SUCCINIC-CYTOCHROME c REDUCTASE ACTIVITY. R. K. Burkhard and D. E. Green. University of Wisconsin. Madison, Wisconsin.

Järnefelt, et al. (Biochim. Biophys. Acta 29, 123, 1959) have described a method for the fragmentation of the succinic dehydrogenase complex (SDC) which yields a red particle (R_2), the prosthetic flavin of which is predominantly that of the succinic dehydrogenase. By modification of Järnefelt's procedure an analogous particle can be prepared which has much higher succinic-cytochrome c reductase activity, contains essentially none of the flavin of DPNH dehydrogenase, and is antimycin sensitive. The rate of reduction of cytochrome c by succinate is approximately equal to that of the reduction of ferricyanide by succinate. Studies on the extent and rate of reduction of the cytochromes and of coenzyme Q in the particle will be discussed in relation to the sequence of components in the chain.

10 *Biochemistry*

SOLUBILIZATION OF THE PARTICULATE FRACTION OF A PYRUVATE OXIDASE SYSTEM. F. Robert Williams* and Lowell P. Hager, Chemistry Department, Harvard University, Cambridge, Massachusetts.

A pyruvate oxidase system which requires both a soluble, non-autooxidizable flavoprotein and a cytochrome-containing particulate fraction for the oxidation of pyruvate to acetate with the uptake of molecular oxygen has been described in both Proteus vulgaris and Escherichia coli. The cytochrome-containing particulate fraction is not amenable to classical purification procedures, and attempts to resolve this fraction with lipid solvents has uniformly led to denaturation. Subsequently, the action of various hydrolytic enzymes on the particulate fraction was studied and it was shown that trypsin hydrolysis of the particulate fraction released approximately 50% of the total dry weight in the form of a solubilized, heat labile, non-dialyzable, enzymatically-active material which couples with the soluble fraction to catalyze the oxidation of pyruvate with either oxygen or ferricyanide as the electron acceptor. The solubilized material is denatured by ammonium sulfate fractionation but has been further purified by acid precipitation and by calcium phosphate gel fractionation. Reduction of the purified material either enzymatically with the soluble flavoprotein and substrate or with dithionite clearly indicates the presence of a cytochrome B_1.

11 *Biochemistry*

ENZYMATIC OXIDATION OF REDUCED NICOTINAMIDE RIBONUCLEOSIDE. H.G. Williams-Ashman and S. Liao*. Univ. of Chicago

A soluble enzyme catalyzing the oxidation of reduced nicotinamide ribonucleoside (NRH) by menadione was partially purified from rat kidney. The enzyme does not react with DPNH, TPNH, acetylpyridine DPNH and NMNH. Few other quinones act as electron acceptors, and the enzyme is inert towards GSSG, thioctic acid, methylene blue and cytochrome c. The latter is reduced enzymatically by NRH in the presence of trace amounts of menadione. The enzyme is inhibited by mercurials, atabrine, chlorpromazine and low levels of estradiol. It is inactivated by treatment with acid ammonium sulfate and activity is restored by FAD or FMN, but not by riboflavine. It is much less sensitive to dicoumarol than other soluble kidney enzyme(s) which catalyze the oxidation of DPNH by menadione. The menadione-dependent oxidation of NRH proceeds rapidly in the soluble fraction of liver, heart, kidney and seminal vesicle, but relatively slowly in muscle, prostate and brain.

12 *Biochemistry*

MAGNETIC TITRATION OF CYTOCHROME OXIDASE. Takashi Yonetani (intr. by A. N. Richards), Johnson Foundation, University of Pennsylvania, Philadelphia, Pa.

The magnetic susceptibility change during the reaction of cytochrome oxidase with oxygen was investigated by using a sensitive magnetic susceptometer combined with a regenerative rapid flow system (Brill et al.,Rev.Sci.Instr. 29:383,1958). The purified oxidase from beef heart was diluted with Emasol-phosphate (Yonetani,J.Biol.Chem.,in press), and concentration data were arbitrarily calculated by $\Delta\chi_M^{445m\mu}=91$. The O_2 concentration required to cause the magnetic susceptibility change at 100 millisec. after mixing of the reduced cytochrome oxidase has been evaluated by rapidly mixing the enzymatically or chemically reduced oxidase preparation with known initial O_2 concentrations. Addition of O_2 for a fixed [oxidase] (15 and 30 μM) gives an increase of volume magnetic susceptibility (ΔK) and the graph of ΔK versus $[O_2]$ consists of two straight lines that intersect at a point at which [oxidase] is equal to 1.2 times the oxidizing equivalents added ($4[O_2]$). This agrees well with the spectrophotometric results (Chance and Yonetani, Fed.Proc. 18:202,1959). Both the magnetic and spectroscopic data are difficult to reconcile with the possibilities that a) a, and a copper atom of the oxidase can all donate electrons to oxygen. The molar magnetic susceptibility increment ($\Delta\chi_m$) is equal to $+2{,}500 \times 10^{-6}$ emu./mole.

13 *Biochemistry*

IDENTIFICATION, BY ELECTRON PARAMAGNETIC RESONANCE SPECTROSCOPY, OF FREE RADICALS GENERATED FROM SUBSTRATES BY PEROXIDASE. I. Yamazaki* and H. S. Mason, Univ. Oregon Medical School, and L. Piette*, Varian Associates, Palo Alto.

Free radicals of hydroquinone and ascorbic acid have been detected during peroxidatic oxidations, with a Varian V-4500 spectrometer. Hydroquinone free radical, formed in the peroxidase reaction at pH 4.8, showed hyperfine structure identical to that of autoxidatively-formed p-semiquinone at slightly alkaline pH. The free radical from ascorbic acid oxidation had a sharp doublet structure, g = 2.0034, at pH 4.8 and 6.0, with a splitting of 1.7 gauss. Rate studies at the steady state produced by a flow technique showed that the steady state concentration of free radical is proportional to the square root of enzyme and ascorbic acid concentration, and independent of H_2O_2 concentration within the limits of our experimental conditions.

14 *Biochemistry*

EFFECT OF CHOLATE ON CYTOCHROME c OXIDASE. Arlene P. Martin* and Elmer Stotz, Dept. of Biochemistry, School of Medicine and Dentistry, University of Rochester, Rochester, N. Y.

Cytochrome c oxidase prepared essentially as described by Smith and Stotz (J. Biol. Chem. 209:819, 1954) requires some cholate and $(NH_4)_2 SO_4$ to solubilize the preparation. High concentrations of cholate affect the properties of the oxidase. Enzymatic activity, as measured by the rate of oxidation of leuco 2,6 dichlorobenzenoneindo-3'chlorophenol in the presence of cytochrome c, is inhibited by the addition of cholate at a final concentration of 0.4%. The site of inhibition may be between cytochrome c and the oxidase or between the oxidase and the terminal reactions. Spectrophotometric recordings indicate that even in the presence of 4% cholate reduced oxidase readily combines with CO. This suggests that the ability of the enzyme to combine with CO, and therefore with O_2, is not affected. The appearance of a prominent shoulder at approximately 444 mμ in the presence of CO, however, is abolished and a single maximum at 430 mμ is evident. The rate of reduction of the oxidase by dithionite is decreased by high concentrations of cholate and the reoxidation of the enzyme also is affected. The data suggest that cholate may affect both the reduction and reoxidation of the oxidase, but not affect its ability to combine with O_2.

15 *Biochemistry*

EFFECTS OF IONS ON THE DPNH OXIDASE OF PLANT MITOCHONDRIA. D. P. Hackett, Dept. of Biochemistry, Univ. of California, Berkeley, Calif.

The DPNH oxidase activity of particles isolated from sweet potato roots can be stimulated five- to ten-fold by a variety of inorganic ions, at concentrations ranging from 10^{-4} M to 10^{-2} M. Divalent cations are more effective than monovalent cations, and maximum oxidative rates are obtained in the presence of sucrose (>0.4 M). External electron acceptors, such as dichlorophenolindophenol and cytochrome c, give comparable stimulations. The salts, particularly divalent cations, and cytochrome c also cause a rapid increase in the optical density of the mitochondrial suspension("contraction"), but this is less pronounced in the presence of sucrose. The results suggest that this structural effect is related to, but not directly responsible for, the increased DPNH oxidation. These observations may throw some light on the problems of respiratory control and salt respiration in plants. (Supported in part by a grant from the National Science Foundation.)

16 *Biochemistry*

DPNH DEHYDROGENASE(S) FROM EXTRACTS OF VENOM DIGESTED HEART MUSCLE PARTICLES. Tsoo E. King and Robert L. Howard*. Oregon State College, Corvallis.

Digestion of the Keilin-Hartree heart muscle preparation with very low concentrations of boiled snake venom in systems similar to those used by Ball, Ambe, and co-workers, rapidly destroyed its DPNH oxidase activity. Under these conditions a negligible activity of soluble DPNH dehydrogenase which was tested with 2,6-dichlorophenolindopehnol (DCP) emerged. However, the liberation of the activity was observed with higher concentrations of venom. About 50 fold increase in the specific activity was observed by fractionation of the supernatant fraction of the venom digest with conventional methods. Further purification on DEAE cellulose yielded two distinct fractions which possessed the same behavior so far tested. The fresh preparation catalyzed the oxidation of 3×10^{-2}, 2×10^{-2}, and 2×10^{-4} M DPNH per mg. of protein per ml. per min. when ferricyanide, DCP, and methylene blue respectively were used as electron acceptor. The activity with cytochrome c or lipoic acid was less than 1 per cent of that with ferricyanide in contrast to the Keilin-Hartree preparation which exhibited strong activity of lipoic dehydrogenase. The present enzyme(s) may be different from Straub's diaphorase and DPNH-cytochrome c reductase in view of the different specificities for electron acceptors and other properties.

17 *Biochemistry*

LIPID COMPLEXES OF CYTOCHROME c. Melvin Reich* and W.W.Wainio. Bur. Biol.Res. and Dept.Physiol. and Biochem.,Rutgers-The State Univ., New Brunswick,N.J.

Cytochrome c and a total lipid extract, both obtained from beef heart, combine between pH 2-5 to form an insoluble complex. Fractionation of the total lipid indicates that the lipid which is precipitated (30% of the total phosphorus) consists mainly of phosphatidylethanolamine. The phospholipid-cytochrome c gram ratio is approximately 4:1 at pH 5. Solubilization of the complex is accomplished with deoxycholate (0.2% at pH 8.6). The ultraviolet and visible spectrum of the complex is essentially the same as that of cytochrome c. Similar complexes may be prepared with egg cephalin and phosphatidylserine, but not with purified lecithin. The ability of many phospholipids to restore the enzymatic activity of a phospholipid-free cytochrome c oxidase preparation is believed to depend on the formation of a phospholipid-cytochrome c complex which remains in solution at the pH of the enzyme assay (6.0). Added deoxycholate reduces this activation by competitively inhibiting the formation of the complex. Dehydrocholate which does not solubilize the complex has no effect on the cytochrome c oxidase activity.

18 *Biochemistry*

A NEW GLUTATHIONE-DISULFIDE TRANSHYDROGENASE. John F. Thompson*, Simon Black, and Blondel Hudson*. Natl. Insts. of Health, Bethesda, Md.

An enzyme has been found in yeast extracts which catalyzes the reduction of a number of disulfides by glutathione, $2\ GSH + (RS)_2 \rightleftarrows (GS)_2 + 2\ RSH$. It has been purified 25-fold and completely separated from glutathione reductase. When coupled with glutathione reductase it forms a convenient system for the reduction of disulfides by TPNH, GSH then acting catalytically. This enzyme differs from the homocystine specific transhydrogenase of Racker (J. Biol. Chem. 27, 867 (1955)) in its lack of specificity; L- or D-cystine, lipoate, mercaptoethanol disulfide, as well as homocystine are all reduced at comparable rates in this system. The enzyme is very powerful in yeast having an activity in crude extracts equivalent to that of glutathione reductase. This transhydrogenase is distinct from another TPNH-linked disulfide reduction system in yeast which is a part of the L-methionine l-sulfoxide reducing system (Federation Proc. 17, 191 (1958)). The latter does not require GSH. A point of additional interest is that yeast extracts contain non-dialyzable, ammonium sulfate-precipitable substances, apparently disulfides of high molecular weight, which serve as substrates for the disulfide reducing enzymes.

XUM

19 *Biochemistry*

MITCHONDRIAL OXIDATION OF EXOGENOUS DPNH BY AN α-GLYCEROPHOSPHATE CYCLE. E. I. Ciaccio, and D. L. Keller (intr. by E. E. Howe.) Merck Institute for Therapeutic Research, Rahway, New Jersey.

The oxidation of α-glycerophosphate (GP) occurs at appreciable rates in preparations of mitochondria and homogenates. The levels of GP oxidase in various animal tissues and the kinetics in intact mitochondria indicate the importance of this enzyme in GP metabolism. Data obtained anaerobically with homogenates and structurally intact tissues in situ indicate that GP is one of the major products of carbohydrate metabolism in normal tissues. The occurrence of a functional cycle for the terminal oxidation of DPNH involving GP is indicated by the following results. Freshly isolated liver mitochondria do not oxidize added DPNH as measured spectrophotometrically. If either GP or cytoplasmic GP-dehydrogenase is added separately no change occurs. On addition of both materials, however, a rapid oxidation of DPNH is observed which is due to the GP cycle involving cytoplasmic GP dehydrogenase, mitochondrial GP oxidase and their intermediate products, dihydroxyacetone phosphate and GP. In addition, data on carbohydrate metabolism in whole homogenates indicate that the GP-cycle is functional under aerobic conditions. (Supported by CCNSC, NCI, NIH Contract #SA-43-ph-1886.)

20 *Biochemistry*

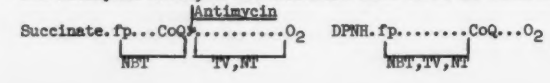
STUDIES ON THE MECHANISM OF ENZYMATIC HYDROGEN TRANSFER REACTIONS. R. H. Baker, Jr.*, R. Suzue* and H. R. Mahler. Dept. of Chem., Indiana Univ., Bloomington.

The initial rates of many enzymatic reactions obey the empiric relationship (1) $v_o^{-1} = \phi_0 + \phi_1 (A)^{-1} + \phi_2 (B)^{-1} + \phi_{12} (A)^{-1}(B)^{-1}$ where (A) and (B) are the stoichiometric concentrations of substrate and co-substrate, coenzyme or inhibitor respectively. It has been shown that application of the steady state approximation to several different mechanisms leads to eq. (1) (Alberty, J. Am. Chem. Soc., 75: 1928, 1953; Dalziel, Acta Chem. Scand. 11: 1706, 1957). We have programmed eq. 1 for the IBM 650: this permits the rapid, efficient and objective calculation of the coefficients. In order to select the most plausible mechanism in the case of H-transfer reactions an useful criterion is provided by the kinetic H/D effect, i.e. the effect on the various coefficients of substituting D for H in A or B. Different and distinctive results are predicted for various mechanisms. The glucose, yeast alcohol and liver alcohol dehydrogenase reactions have been studied. Significant isotope effects have been observed for different steps in the postulated reaction sequence, including the one for the binding of DPNH by the enzyme. A comparison of the results obtained by various kinetic and equilibrium binding methods will be presented. (Supported by grants from ONR and the American Cancer Soceity.)

21 *Biochemistry*

TETRAZOLIUM REDUCTION BY MITOCHONDRIAL PREPARATIONS; PARTICIPATION OF COENZYME Q. A. L. Smith and R. L. Lester (intr. by L. J. Teply.) Univ. of Wisconsin, Madison, Wisconsin.

The pathway of reduction of tetrazolium violet (TV), neotetrazolium (NT) and p-nitro-blue tetrazolium (NBT) by succinate and DPNH has been studied with beef heart mitochondria and with acetone-extracted mitochondria which are devoid of coenzyme Q (CoQ). After appropriate incubation, a mixture of formalin and Triton X-100 (pH 3.5) was added to stop the reaction and render the solution optically clear, obviating the necessity for extracting the colored formazans with organic solvents. Succinate-tetrazolium reductase activity was sharply decreased by acetone extraction; full activity was restored by either the addition of CoQ or by CoQ plus accessory lipids. The restored activity was sensitive to antimycin for NT and TV, insensitive to antimycin for NBT. DPNH-tetrazolium reductase activity was either increased (NT,TV) or not greatly altered (NBT) by acetone extraction and no effects of CoQ were observed. The principal points of interaction of these tetrazolium salts with the electron transport system, particularly with respect to the CoQ and antimycin locii, would therefore seem to be as follows:



22 *Biochemistry*

BIOLOGICAL ACTIVITY OF A PURIFIED CYTOCHROME c_1 PREPARATION. Robert P. Glaze* and Martin Morrison, Dept. of Biochemistry, School of Medicine and Dentistry, University of Rochester, Rochester, N. Y.

Cytochrome c_1 was prepared in an active form by butyl alcohol-cholate treatment of a trypsin digested, Keilin-Hartree extract of pig heart. The yield of active material can be increased by the use of thioglycolic acid during the butyl alcohol treatment. Cytochrome c_1 is obtained in the reduced form and no other hemoproteins can be detected in this preparation. Cytochrome c_1 is oxidized slowly by a purified preparation of cytochromes a and a_3 (1.1 μmoles c_1 oxid./min./μmole heme a). The addition of one mole of cytochrome c per mole of hemin a increases this rate twelvefold. The cytochrome c_1 preparation contains no succinate-cytochrome c_1 reductase activity, but the cytochrome c_1 is reduced by a succinate-cytochrome c reductase preparation. The DPNH-cytochrome c_1 reductase activity of this preparation is 5.63×10^{-3} μmoles c_1 reduced/min./mg. protein, and the DPNH-cytochrome c reductase activity is 78×10^{-3} μmoles c reduced/min./mg. protein. TPNH does not reduce either cytochrome c or cytochrome c_1 in this preparation. These results indicate that, in this preparation, DPNH reduction of cytochrome c is not mediated by cytochrome c_1.

23 *Biochemistry*

D(-)β-HYDROXYBUTYRATE DEHYDROGENASE IN THE OXIDATION OF EXOGENOUS DPNH BY MITOCHONDRIA. Thomas M. Devlin and Bernice H. Bedell (intro. by W. W. Umbreit), Merck Institute for Therapeutic Research, Rahway, New Jersey.

Intact rat liver mitochondria oxidize exogenous DPNH at a negligible rate presumably because the mitochondrial membrane is impermeable to DPNH. It was observed that catalytic quantities (1.0 μmole) of acetoacetate or D(-)β-hydroxybutyrate (BOH) stimulated the oxidation of DPNH with a concomitant phosphate uptake (P/O = 1.5 to 2.7). Other mitochondrial substrates had no effect on DPNH oxidation. It is proposed that BOH dehydrogenase catalyzes the extramitochondrial reduction of acetoacetate to BOH which is reoxidized within the mitochondrion by the dehydrogenase coupled to the cytochrome chain. The extramitochondrial activity of BOH dehydrogenase was measured spectrophotometrically with intact mitochondria in the absence of cytochrome c and respiratory inhibitors. No appreciable extramitochondrial activity of other dehydrogenases was observed. In manometric studies, added cytochrome c and DPN relieved the amytal inhibition of BOH oxidation with intact mitochondria but had only a slight effect on the amytal inhibited oxidation of other substrates. The results suggest that liver mitochondria contain two spatially separated BOH dehydrogenases, one functioning externally and the other internally. (Supported by CCNSC, NCI, NIH Contract #SA-43-ph-1886.)

24 *Biochemistry*

A CELL-FREE NITRITE OXIDIZING SYSTEM FROM NITROBACTER. M.I.H. Aleem* and A. Nason. McCollum-Pratt Institute, The Johns Hopkins University, Baltimore, Maryland.

A cytochrome-containing particle designated as nitrite oxidase, which catalyzes the oxidation of nitrite to nitrate by O_2, has been obtained from the obligately chemoautotrophic bacterium Nitrobacter agilis. Nitrite oxidase activity is confined to the red particles collected by differential high-speed centrifugation of sonicated cells. Nitrite or dithionite addition to the cell-free particles (or to intact cells) resulted in absorption maxima at 550 and 520 mμ corresponding to the alpha and beta peaks of a cytochrome c-like component, and at 585-590 and 438 mμ representative of a cytochrome of the a-type. Neither DPNH, succinate or lactate produced these spectra; nor could peroxidase or catalase activities be detected. The cytochrome a-like peaks appear only in particulate cell-free fractions possessing nitrite oxidase activity, while soluble fractions lacking activity show smaller absorption maxima at 550 and 520 mμ and practically none at 585-590 mμ. Nitrite oxidase is specifically reactivated by iron following cyanide dialysis, and is inhibited by azide, antimycin A and p-chloromercuribenzoate. Added FAD or FMN had no effect. It is suggested that nitrite oxidase activity involves a sequence of electron transport from nitrite to O_2 via cytochrome c- and cytochrome a-like components.

020. BIOLOGICAL OXIDATION–Krebs Cycle

25 *Biochemistry*

PROPERTIES OF CAULIFLOWER MITOCHONDRIA. W. D. Bonner, Jr. and T. Ito (Intr. by D. R. Goddard), Johnson Foundation, University of Pennsylvania, Philadelphia, Pennsylvania.

The preparation from higher plants of mitochondria which show a respiratory response to ADP has not been previously accomplished. For various reasons highly buffered extracting media always have been employed; unfortunately, such media extract a considerable amount of cytochrome c. Mitochondria prepared in well buffered media oxidize all the TCA acids and DPNH. Such oxidations require added cytochrome c and the fatty acid oxidation cofactors. Mitochondria prepared from cauliflower by methods to be described, only oxidize succinate, malate, α-ketoglutarate, and DPNH; they do not require any organic cofactors other than ADP. The addition of succinate to these mitochondria results in a very low rate of oxygen uptake; the rate is increased up to 10-fold on the addition of ADP. Low temperature spectra of substrate-reduced mitochondria show maxima at 597, 562, 557, 554, and 549 mμ in a ratio of 1:0.1:0.7:0.9:1.3. Treatment with antimycin-A in the presence of substrate gives a three banded spectrum with maxima at 563, 558 and 555 mμ. Substrate reduction in the presence of cyanide shows the 597 and 549 mμ maxima only. Reduction with hydrosulfite reveals a large amount of non-enzymatically reducible cytochrome.

26 *Biochemistry*

ELECTRON TRANSFER BY GLUTATHIONE-MENADIONE COMPLEX (THIODIONE) IN TERMINAL REDUCTIONS. Walter J. Nickerson, Giuseppe Falcone*, and George Strauss*. Inst. of Microbiology, Rutgers Univ., New Brunswick, N. J.

The formation of a complex between menadione and glutathione is briefly mentioned by Fieser and Fieser in their textbook of Organic Chemistry. The yellow complex is formed rapidly in ethanolic solution and has been obtained in chromatographically pure form. The complex (2-methyl, 3-glutathionyl-1,4-naphthoquinone = thiodione) is insoluble ir ethanol, acetone, or hexane, but is highly soluble in water, and is stable in the dark at room temperature; it is rapidly destroyed under alkaline conditions. Thiodione is very rapidly reduced enzymatically by DPNH or TPNH, or chemically by dithionite or titanous chloride (E'_o = -0.010 volt). In solution, the colorless reduced thiodione is easily oxidizable by air, or by selenite (with the production of metallic selenium). The addition of thiodione, together with glucose-6-phosphate and TPN, fully restored catalytic activity to a dialyzed enzyme preparation from baker's yeast capable of selenite reduction. (Supported by a grant from National Institutes of Health.)

27 *Pharmacology*

ON THE SITE OF ACTION OF A SUCCINOXIDASE INHIBITOR FROM HYDRA. Edward S. Kline* and Vaman S. Waravdekar. Biochemistry Branch, Armed Forces Institute of Pathology, Wash., D. C.

Previous studies in this laboratory have shown that Hydra produces a powerful inhibitor of the succinoxidase system. The inhibitor, a protein, appears to be a component of the toxin in the nematocysts of this animal. By the criteria used, the inhibition produced by this protein is of a specific nature. At concentrations which inhibit essentially all of the succinoxidase activity, there is no inhibition of cytochrome-c-oxidase and succinic dehydrogenase activity in the assay systems we have employed. Even at considerably higher concentrations there is little or no inhibition of these two systems. The succinate-cytochrome-c-reductase system is inhibited, but only at high concentrations of the inhibitor. These and other aspects of the nature of the inhibition will be discussed. Studies are in progress which may further elucidate the site and mechanism of action of this inhibitor from Hydra.

1 *Physiology*

CITRATE AND TRICARBOXYLIC ACID CYCLE ENZYMES IN THE CATERPILLAR OF THE SOUTHERN ARMYWORM, PRODENIA ERIDANIA. L. Levenbook. Natl. Insts. of Health, Bethesda, Md.

Among a variety of organic acids in the larval hemolymph (blood) of the Southern Armyworm citrate is a major component, with an average concentration of 0.02M. Citrate and other TCA cycle intermediates are oxidized to completion by washed larval mitochondria prepared from the entire internal tissues in 0.25M sucrose - 0.01M EDTA. The endogenous mitochondrial Qo_2 (per mg protein) is ~10.1 and this increased 3-4 fold in the presence of 1 μM citrate and appropriate cofactors. A study has been made of the TCA cycle and related enzymes in P. eridania. Tissue acetone powder extracts were used where possible, otherwise isolated mitochondria or individual tissue homogenates were employed. The following enzymes were found to be active: aceto-CoA-kinase; condensing enzyme; citrate cleaving enzyme; aconitase; TPN-linked isocitric dehydrogenase; α-ketoglutarate dehydrogenase; succinic dehydrogenase; fumarase; DPN-linked malic dehydrogenase; TPN-linked "malic enzyme"; oxaloacetate decarboxylase. No evidence could be found for the presence of either isocitratase or malic synthetase. These findings suggest, but clearly do not prove, that citrate in the insect may be both synthesized and oxidized by typical known pathways.

2 *Biochemistry*

DINITROPHENOL-INDUCED STIMULATION OF DARK FIXATION OF CO_2 IN CHLORELLA. Mary L. Stiller (intro. by H. Gaffron) Dept. of Biochemistry (Fels Fund), University of Chicago.

2,4- Dinitrophenol stimulates O_2 consumption and CO_2 production in cells of Chlorella in the dark. These effects are accompanied by a much larger stimulation of $C^{14}O_2$ incorporation into the soluble fraction of the cells. Chromatography and autoradiography show malate, citrate, aspartate, glutamate, hexose and a phosphorylated acid among the labeled products. DNP does not alter the percentage of the total activity incorporated in malate or aspartate but reduces the percentage found in citrate while increasing that found in glutamate. Smaller shifts occur in phosphorylated acid, hexose and a third amino acid. Stimulation of CO_2 fixation by DNP is shown by increases in pool size and specific activity of the labeled compounds. This suggests that DNP stimulates respiration both in intensity (increased O_2 uptake, higher specific activity) and in capacity (larger pools of krebs cycle intermediates.) Although the stimulation of CO_2 fixation by DNP is not entirely expected the observations can be accounted for by a mechanism to be presented.

3 *Biochemistry*

ION REQUIREMENTS FOR OBTAINING AND STABILIZING ISOCITRIC DEHYDROGENASE FROM A MARINE BACTERIUM. R.A. MacLeod, A. Hori* and S.M. Fox*. Fisheries Research Board of Canada, Vancouver.

Cells of a species of marine bacterium contain an isocitric dehydrogenase which is TPN linked, requires Mg^{++} and is non-specifically activated by a number of inorganic salts (optimum ionic strength .3 to .4). When cells were washed and suspended in .05 $MgCl_2$ no isocitric dehydrogenase could be demonstrated in the extract prepared, although all other tricarboxylic acid cycle enzymes could be shown to be present. Failure to detect the enzyme was traced to the presence of halide as the sole anion in the solution used to suspend the cells. Sulfate or phosphate salts gave rise to an active enzyme. These relations applied to extracts prepared by sonic treatment, alumina grinding and lysis by versene and lysozyme. Tested under optimum conditions the specific activity of isocitric dehydrogenase in the extract was found to be proportional to the ionic strength of the solution used to suspend the cells (maximum activity at .4 to .5 μ). In the presence of low concentrations of an anion giving rise to an active enzyme, halide salts were found capable of contributing to these ionic strength requirements. Mg^{++} was required in the extract to maintain the activity of the enzyme on storage at 4°C.

4 *Biochemistry*

PROPERTIES OF PYRUVATE AND α-KETOGLUTARATE DEHYDROGENATION COMPLEXES OF ESCHERICHIA COLI. Lester J. Reed and Masahiko Koike.* Clayton Foundation Biochemical Institute and Dept. of Chemistry, University of Texas, Austin.

CoA- and DPN-linked pyruvate and α-ketoglutarate(KG) dehydrogenation systems have been isolated from extracts of E.coli (Crookes) as multienzyme units(PDC and KGDC) with molecular weights of 4.8 and 2.4 million, respectively. It appears that the complexes may be aggregates of a smaller unit. PDC and KGDC contain, respectively, 40 and 11 moles of bound lipoic acid and 13 and 10 moles of FAD per mole of protein. Release and reincorporation of bound lipoic acid and FAD showed that both cofactors are required for oxidation of pyruvate and KG with DPN as electron acceptor, and for oxidation of pyruvate with free lipoic acid (or lipoamide) as electron acceptor. FAD, but not bound lipoic acid is required for dihydrolipoic dehydrogenase activity of both complexes as measured with free dihydrolipoic acid. Neither cofactor is required for oxidation of pyruvate and KG with ferricyanide as electron acceptor, nor for dihydrolipoic transacetylase activity of PDC as measured with free dihydrolipoic acid. It appears that the site of ferricyanide action is at the "aldehyde-thiamine pyrophosphate" level. The flavin in the complexes is reduced on addition of both pyruvate and CoA or KG and CoA, respectively, and reoxidized on addition of lipoic acid.

5 *Biochemistry*

METABOLISM OF HYDROXYPYRUVATE. Jerry L. Hedrick* and H. J. Sallach. Dept. of Physiological Chemistry, Univ. of Wisconsin Medical School, Madison.

The metabolism of C^{14}-labeled hydroxypyruvate by various tissue preparations has been investigated. Oxidative studies using washed residues from rat liver were carried out in the Warburg apparatus. Since it has been shown that pyruvate oxidase can catalyze the oxidation of hydroxypyruvate (Jagannathan and Schweet, J. Biol. Chem. 196:551, 1952), both hydroxypyruvate and pyruvate were studied for comparative purposes. It was found that DPN, ATP, and TPN had no effect on the oxygen uptakes with hydroxypyruvate, but that DPN was essential for pyruvate oxidation. In the presence of arsenite, pyruvate oxidation was completely inhibited but the oxidation of hydroxypyruvate was unaffected. Hydroxypyruvate is readily autoxidized and decarboxylated under the experimental conditions employed. Due to this, non-enzymatic controls were carried out routinely. The enzymatic oxidation of hydroxypyruvate without added DPN and the lack of inhibition by arsenite suggest that hydroxypyruvate may be oxidized by an enzyme system other than pyruvic oxidase. With either homogenates or washed residues, C^{14}-labeled hydroxypyruvate gives rise to several radioactive compounds as shown by chromatographic procedures. The identification of these products is currently in progress. (Supported by grants from USPH and WARF.)

6 *Biochemistry*

PURIFICATION AND PROPERTIES OF MALATE SYNTHETASE. G.H. Dixon, H.L. Kornberg and Patricia Lund (Intr. by D.A. Scott). M.R.C. Cell Metabolism Unit, Dept. of Biochemistry, Univ. of Oxford.

Malate synthetase,which catalyzes the formation of malate by condensation of acetyl CoA and glyoxylate, has been purified 200-300 fold from baker's yeast and from glycollate-grown Pseudomonas ovalis Chester. The rate of decrease of optical density at 232 mμ consequent upon cleavage of the thioester bond of acetyl coenzyme A in the presence of glyoxylate was used to follow the reaction. Km for glyoxylate was 9.3×10^{-5}M (yeast) and 6.3×10^{-5}M (Pseudomonas) while the Km for Acetyl CoA was $< 1 \times 10^{-5}$M. The enzymes required Mg^{++} for activity, were optimally active at pH 8.5, and were competitively inhibited by oxalate, fluoroacetate and glycollate. The purified enzymes were specific for glyoxylate and did not catalyse the cleavage of acetyl CoA in the presence of oxaloacetate, or any other keto acid or aldehyde tested. In the presence of glyoxylate, there was no reaction with any other thiolester except fluoroacetyl CoA which reacted at approx. 25% of the rate observed with acetyl CoA. The equilibrium strongly favours malate synthesis and no reversal of the reaction was detected. Studies on the mechanism of action indicated that malyl CoA was not an intermediate nor was the acetyl group transferred to the enzyme protein prior to condensation with glyoxylate.

7 *Biochemistry*

PYRUVATE CARBOXYLASE. M. F. Utter and D. B. Keech*. Dept. of Biochem., Western Reserve U., Cleveland, Ohio.

An enzyme system obtained from avian liver mitochondria forms oxalacetate from pyruvate and CO_2. This reaction is not related to the activity of malic enzyme or PEP carboxykinase but rather constitutes another mechanism for the formation of dicarboxylic acids by CO_2 fixation in liver. The enzyme(s) has been purified to some extent from acetone dried mitochondria and the reaction found to be inhibited by avidin, suggesting the participation of biotin. In addition to pyruvate and CO_2, oxalacetate synthesis requires ATP, Mg and acetyl CoA. Stoichiometric studies and experiments with $C^{14}O_2$, pyruvate-C^{14} and acetyl-1-C^{14} CoA indicate that oxalacetate arises from CO_2 and pyruvate and not from acetyl CoA. The role of the latter substance has not been elucidated but the rate of CO_2 fixation with acetyl CoA in the absence of pyruvate is very slow compared with the overall rate of oxalacetate formation. This observation along with other evidence does not support the view that acetyl CoA participates by way of carboxylation to malonyl CoA, followed by transcarboxylation of the latter compound with pyruvate.

8 *Biochemistry*

EVIDENCE FOR DITHIOL PARTICIPATION IN DIHYDROTHIOCTYL DEHYDROGENASE REACTION. R. L. Searls. (intr. by L. P. Zill). Gerontology Branch, National Heart Institute, and Baltimore City Hospitals, Baltimore 24, Maryland.

Dihydrothioctyl dehydrogenase isolated from trypsin digest of α-ketoglutaric dehydrogenase is a flavoprotein with diaphorase activity (Proc. Nat. Acad. Sci., 45, 698, 1959). The thioctyl dehydrogenase activity is inhibited by preincubation of the enzyme with DPNH and arsenite or Cd^{++}. Partial reversal is obtained by 2,3-dimercaptopropanol but monothiols, even at much higher levels, have no effect. The diaphorase activity, however, is increased, as much as five-fold in some preparations, by arsenite or Cd^{++}. The sensitivity to dithiol inhibitors and the requirement for DPNH in the preincubation mixture implicate a vicinal dithiol as an essential red-ox group (besides FAD) in the enzyme. The diaphorase activity is independent of the dithiol in the enzyme. These results may be related to the observation that although Straub diaphorase and the above thioctyl dehydrogenase are indistinguishable on the basis of several criteria (spectral analysis of oxidized and reduced enzyme pH optima and K_M with thioctic acid or amide, and electrophoretic mobility at pH 6.5 and 7.2) the ratio of dehydrogenase to diaphorase activities are different and vary from preparation to preparation. However, this ratio is nearly constant in the presence of Cd^{++}.

9 *Nutrition*

THE CITRIC ACID CYCLE IN CERTAIN ALTERATIONS OF ACID-BASE BALANCE IN HUMAN SUBJECTS. Wilbert Gamble*, James M. Orten, and Arthur H. Smith. Department of Physiological Chemistry, Wayne State University College of Medicine, Detroit.

One of the well-established responses in human subjects to shifts in acid-base balance following alkali administration is a concomitant increase in the excretion of citric acid in the urine. The question arises as to the origin of the citrate, whether by way of the "Citric Acid Cycle" and, if so, whether other members of the cycle also appear in the urine in increased amounts after alkali administration. The present study of this problem employed young normal adults. Complete "24-hour" urine samples were collected during a control period and then after the ingestion of $NaHCO_3$ (20 gm./day) or NH_4Cl (2 gm./day). The citric acid cycle members were determined quantitatively in aliquots of the urine samples by a modification of the silica gel chromatographic technique employed in this laboratory. The pH and total titratable acidity of the samples were also determined. The results indicate that of the cycle acids, only citric acid is increased in response to alkali administration. The amounts of the other cycle acids appear to be somewhat decreased. Certain other non-cycle acids are increased, however. The reverse of this pattern appears to occur in the NH_4Cl period. The implications of these results will be discussed.

021. BIOLOGICAL OXIDATION—
Oxidative Phosphorylation

1 **Physiology**

THE EFFECT OF RIBOFLAVIN DEFICIENCY AND GALACTOFLAVIN FEEDING ON THE MITOCHONDRIAL FLAVIN CONTENT, OXIDATIVE PHOSPHORYLATION, ATPase and ATP-P_i EXCHANGE. Stanley L. Lamberg, Robert E. Beyer and Arthur Neyman (intr. by D. Rapport), Physiology Dept., Tufts Univ. School of Medicine, Boston, Mass.

Lindberg et al. (Acta Chem. Scand. 12:598, 1958) have postulated the participation of flavin in respiratory chain phosphorylations. Löw (Biochim. Biophys. Acta 32:1, 1959) has presented evidence in favor of this concept. We have confirmed Löw's experiments on the effects of atebrin in mitochondrial ATPase reactions. We have attempted to obtain information on flavin participation by studying oxidative phosphorylation and related reactions in mitochondria from animals deprived of dietary riboflavin for one to 14 weeks as well as on animals fed a galactoflavin enriched riboflavin diet. No differences were observed in phosphorylative capacity and ATP-P_i exchange rates in mitochondria from either experimental groups, in spite of a considerably lowered mitochondrial flavin content. The DNP stimulated ATPase activity from riboflavin-deficient animals was increased. Atebrin, at low concentrations, stimulated DNP-ATPase to a greater degree in galactoflavin-fed preparations than in mitochondria from controls. These data indicate a function for flavin in mitochondrial DNP-ATPase but do not indicate a function in oxidative phosphorylation or ATP-P_i exchange.

2 **Physiology**

STUDIES ON A SOLUBLE ATPase FROM RAT-LIVER MITOCHONDRIA. Robert E. Beyer[1], Dept. of Physiology, Tufts Univ. School of Medicine, Boston, Mass.

Twice-washed rat-liver mitochondria have been treated with sound (10 kc, 10 min.) in the presence of 0.01 M Tris-HCl and 0.25 M sucrose. The supernatant, following 4 hrs. at 105,000 x g, was further treated in the cold with ammonium sulfate and dialyzed against 0.01 M Tris-HCl, pH 7.4. High speed centrifugation yielded a supernatant containing ATPase activity with a pH optimum between 7.4 and 7.6 in the presence of Mg ions. High concentrations of Mg^{++} were inhibitory. 2,4-dinitrophenol (10^{-4}M) stimulated ATPase activity in the presence, but not in the absence, of Mg^{++}. Enzyme activity was linear with enzyme concentration. Atebrin (0.4 mM final concentration), a flavin inhibitor, lowered ATPase activity both in the presence and the absence of dinitrophenol. Substituted quinones, such as coenzyme Q (ubiquinone) and vitamin K_1, which inhibit Mg^{++}-activated ATPase in intact mitochondria, were without effect on the soluble mitochondrial enzyme. FAD appears to have a slight inhibitory effect on the enzyme in the presence of Mg^{++}. It is postulated that the soluble ATPase system functions in the oxidative phosphorylation system of intact mitochondria.

[1] U.S.P.H.S. Senior Research Fellow

3 **Physiology**

EFFECT OF HYPERTHYROIDISM ON OXIDATIVE PHOSPHORYLATION IN MITOCHONDRIA FROM THE HEART AND LIVER. D. A. Piatnek and C. W. Lee (intr. by H. S. Belding), Dept. of Biochem. and Nutr., Univ. of Pittsburgh, Pittsburgh, Pa.

Experimental hyperthyroidism was induced in 200 rats and 5 dogs by the oral feeding of U.S.P. desiccated thyroid powder at 1% and 5% of the diet respectively. Persistent signs of hyperthyroidism were evident in rats within 7 to 10 days and in the dogs within 4 to 6 weeks. Rates of oxidative phosphorylation by sarcosome preparations were measured both by a polarographic technique (Arch.Biochem. & Biophy., 70, 290 (1957)) and by the classical manometric method. In 8 experiments with sarcosomes from thyrotoxic rats P/O ratios of 3.1 ± 0.3 and 2.9 ± 0.3 were obtained with the polarographic and manometric techniques respectively as compared to 3.3 ± 0.3 and 3.0 ± 0.3 from the controls. In agreement with Piatnek and Olson (Fed. Proc., 15, 145 (1956)) ATP and CP were found to be normally distributed in the thyrotoxic rat heart. The rates of oxidative phosphorylation in sarcosome preparations from 5 hyperthyroid dogs, 3 of which demonstrated hemodynamic signs of cardiac failure, were also found to be normal. Significantly depressed rates of oxidative phosphorylation were demonstrated in liver mitochondrial preparations from both thyrotoxic rats and dogs (Maley and Lardy, J. Biol. Chem., 215, 377 (1955)).

4 **Biochemistry**

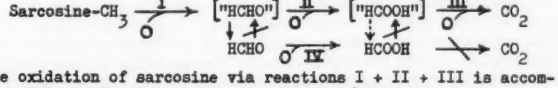
OXIDATIVE PHOSPHORYLATION WITH LIVER MITOCHONDRIA IN ETHIONINE-FED RATS. J. A. Stekol, U. Mody*, E. Bedrak*, S. Keller*, J. Perry*, Inst. for Cancer Research, Philadelphia.

The synthesis of liver DPN and DPNH from nicotinamide was inhibited by ethionine in the diet (Stekol et al., Federation Proc. 18, 331 (1959)). The oxygen consumption and esterification of inorganic phosphate with liver mitochondria of ethionine-fed rats was decreased with pyruvate and α-ketoglutarate as the substrates. With succinate as the substrate, oxidative phosphorylation was not affected by etionine feeding. S-Adenosyl-L-ethionine, added to normal mitochondria, also inhibited the oxidative phosphorylation with pyruvate and α-ketoglutarate as the substrates, while it had no effect on oxidative phosphorylation with succinate as the substrate. S-adenosyl-L-methionine did not affect the oxidative phosphorylation with normal liver mitochondria with pyruvate, α-ketoglutarate, or succinate as the substrate. The livers of ethionine-fed rats contained per gram of fresh tissue markedly less ATP, increased amounts of AMP, and unchanged quantities of ADP. The de novo synthesis of pyridine nucleotide and nucleic acids[1] adenine from glycine-2-C-14 was also markedly reduced in ethionine-fed rat liver. Attempts to reverse the inhibitions induced by ethionine feeding in liver mitochondria by additions of DPN and Co-A will be described. Aided by grants from American Cancer Society and Nat. Cancer Inst.USPHS

5 **Biochemistry**

OXIDATIVE PHOSPHORYLATION IN THE MITOCHONDRIAL METABOLISM OF SARCOSINE AND FORMALDEHYDE. W. R. Frisell, N. C. Sorrell*, and C. G. Mackenzie, Univ. of Colo. School of Med., Denver, Colo.

In the presence of sucrose-suspended mitochondria of rat liver, plus ADP and phosphate, the $-C^{14}H_3$ group of sarcosine is oxidized to HCHO, formate, and CO_2. Exogenous HCHO is oxidized only to formate. Exogenous formate and L-serine are inert. These findings establish the following sequence:



The oxidation of sarcosine via reactions I + II + III is accompanied by a phosphate uptake to give a P/O ratio of 2 for the total sequence. The conversion of exogenous HCHO to formate (reaction IV) also yields a P/O ratio of 2. In the presence of semicarbazide, the rate of oxidation of the sarcosine-CH_3 group doubles, and the accumulation of HCHO increases 6-fold. However, the P/O ratio under these circumstances remains at 2. These and the foregoing results permit a minimum P/O ratio of 2 to be assigned to each of the steps--I, II and III. Dinitrophenol uncouples completely the oxidative-phosphorylation with sarcosine and prevents oxidation of the N-CH_3 group beyond formate. Phosphorylation is also uncoupled by pentachlorophenol (E. C. Weinbach, personal communication).

6 **Biochemistry**

STUDIES ON THE MECHANISM OF THE STIMULATION OF OXIDATIVE PHOSPHORYLATION BY THYROXINE. J. Ramsey Bronk (intr. by W. W. Kielley), Columbia University, New York.

Previously published work (J. R. Bronk, Biochim. et Biophys. Acta 37, 327, 1960) has established that thyroxine will cause an increase in the initial rate of oxidation of succinate and DPNH by submitochondrial particles prepared by sonic disintegration of rat liver mitochondria. Thyroxine also improved the efficiency of the phosphorylation associated with the oxidation of these substrates. It is the purpose of this communication to describe experiments made to elucidate the mechanism responsible for these effects. A series of experiments has established that the stimulation of oxidation and phosphorylation by thyroxine is slight at temperatures of 15°C and below and becomes progressively more marked as the temperature is increased up to 39°C. A second group of experiments has established that dilution of the submitochondrial particle suspension results in a greatly increased stimulation of both oxidation and phosphorylation by thyroxine. These results are consistent with the hypothesis that thyroxine acts on the mechanism responsible for coupling the terminal steps of phosphorylation to the electron transport chain and that the added thyroxine is replacing some unknown natural component lost from the particles. (Supported by a grant from the U. S. Public Health Service)

7 **Biochemistry**

EFFECT OF FATTY ACIDS ON THE ATP-P_i^{32} EXCHANGE REACTION ASSOCIATED WITH OXIDATIVE PHOSPHORYLATION IN RAT LIVER MITOCHONDRIA. A.B. Falcone, E. Shrago and R.L. Mao (Intr. by M.J. Johnson) VA Hosp. and Univ. of Wis., Madison, Wisconsin.

Various saturated and unsaturated fatty acids were found to inhibit the ATP-P_i^{32} exchange reaction associated with oxidative phosphorylation. This inhibition was greatly augmented by blocking the oxidation of the fatty acids by any of the following: 10^{-3}M KCN, 2 x 10^{-3}M NaN_3, 4 x 10^{-3}M amytal, 2 micrograms/ml of antimycin-A, anaerobic conditions. A concentration of 8 x 10^{-5}M myristic acid with 10^{-3}M KCN resulted in a 95% reduction of the amount of exchange carried out by controls. Of the saturated fatty acids studied, greatest inhibitory activity was found with palmitic, myristic and lauric acids. ATPase activity in the presence of myristic acid under conditions of the exchange reaction was negligible except in the concentration range 2.4 x 10^{-5}M to 1.2 x 10^{-4}M where a slight increase was found. No significant changes in absorbancy of reaction mixtures at 520 mμ were noted under conditions where the ATP-P_i^{32} exchange was greatly inhibited. The results suggest that the inhibitory effects of fatty acids on the ATP-P_i^{32} exchange is not dependent on the presence of the reduced state of the various carriers of the respiratory chain subsequent to DPN and that their mode of action may be on some phase of the coupling mechanism other than electron transport.

8 **Biochemistry**

FLUORIDE SENSITIVE OXIDATIVE PHOSPHORYLATION IN A GUINEA PIG BRAIN MITOCHONDRIAL SYSTEM. Harold P. Cohen (intr. by John F. Van Pilsum.) Div. of Neurology, Univ. of Minnesota Medical School, Minneapolis.

Mitochondria isolated in sucrose respire and take up Pi at a slow rate in a system containing Pi, Mg^{++}, crude yeast hexokinase (Sigma Type III) and ATP (ADP or AMP). ΔPi was measured isotopically and colorimetrically. Chromatographic analysis after incubation shows the presence of P^{32} labeled hexose monophosphates. PPi was not found. Respiration and phosphorylation are both almost completely depressed by 10^{-2}M fluoride. 2.5 μmoles of DPN greatly increases the activity of the system and provides for linear O_2 uptake over a 30 minute period. P/O ratios of 2.5 with a QO_2 of 17.5 μmoles/gm/hr have been obtained. TPN and GSH in equivalent amounts are inhibitory. The system is sensitive to DNP, cyanide, pentobarbital, $SO_4^=$ and anaerobiosis. DPNH substitutes for DPN with lower phosphorylative efficiency, but respiration is less sensitive to fluoride. The crude hexokinase contains invertase which provides a possible glucose-fructose substrate. Inactivity with crystalline hexokinase plus glucose may be due, in part, to the presence of $(NH_4)_2SO_4$. It is tentatively concluded that this system may represent phosphorylation coupled to the oxidation of DPNH. Substrate metabolism and fluoride effect will be discussed. (Supported by Grant B-1729, PHS)

9 **Biochemistry**

STUDIES ON THE MECHANISM OF AMINONUCLEOSIDE-INDUCED NEPHROSIS IN THE RAT. II. OXIDATIVE-PHOSPHORYLATION. Paul Bartlett and Leophas Ford*. Edsel B. Ford Inst. for Med. Res., Henry Ford Hosp., Detroit, Mich.

Induction of experimental nephrosis in the rat with small daily subcutaneous injections of 1.5 mg of aminonucleoside, 6-dimethylamino-9-(3'-amino-3'-deoxy-β-D-ribofuranosyl)-purine, per 100 gm body weight per day for a period of 10 days, results in a loss of mitochondrial activity in respect to both substrate oxidation and concomitant phosphorylation when succinate is used as a substrate. Thus, for example, in experiments with mitochondria prepared from the pooled kidneys of 2 rats from each of 6 groups of nephrotic animals, the oxygen uptake per mg mitochondrial nitrogen was 14.4 to 39.1% lower than that observed in parallel experiments conducted with normal rat kidney mitochondria. In general, concomitant phosphorylation, measured in terms of disappearance of inorganic phosphate, was similarly reduced. Loss in activity in respect to oxidation was even more striking when β-hydroxybutyrate was used as a substrate. In 3 experiments with nephrotic kidney mitochondria, acetoacetate formation was 45.4 to 51.7% lower than that observed with normal rat kidney mitochondria. Phosphorylation was not, however, similarly impaired.

10 **Biochemistry**

ENZYMATIC FORMATION OF A REDUCED DERIVATIVE OF VITAMIN K DURING OXIDATIVE PHOSPHORYLATION. Percy J. Russell* and Arnold F. Brodie, Dept. of Bacteriology, Harvard Med. School, Boston, Massachusetts.

Evidence for the role of naphthoquinones as coenzymes in electron transport and in phosphorylation has been obtained with extracts of Mycobacterium phlei. Although the formation of ATP can be demonstrated with a model compound (menadiol monophosphate), neither the reduction nor the phosphorylation of an added naphthoquinone has been demonstrated because the reduced compound is labile to oxidation. The reduced vitamin can be stabilized, however, by substitution of the quinol hydrogens with a variety of chemical groups. This has provided a means of trapping and analyzing the reduced intermediates formed. With preparations capable of oxidative phosphorylation, plus substrate amounts of vitamin K_1 and either DPNH or malate, the reduced vitamin is formed under anaerobic conditions and trapped by acetylation. Lipid solvent extraction and chromatography yield fractions which spectrally resemble synthetic diacetyl vitamin K_1 and which contain organic phosphate. These reduced compounds are not formed in the absence of added vitamin K_1, under aerobic conditions or in the absence of oxidative phosphorylation. The role of these compounds in electron transport and phosphorylation will be discussed.

11 **Biochemistry**

ELUCIDATION OF A FACTOR ESSENTIAL IN OXIDATIVE PHOSPHORYLATION OF A MAMMALIAN SYSTEM. Lemuel D. Wright, Maria Rakowska* and Rosemarie Hanson*. Graduate School of Nutrition, Cornell University, Ithaca, New York.

Biosynthesis of non-saponifiable material from mevalonic acid by whole homogenates of rat liver is directly proportional to the ATP level existing at the time the precursor is added (Wright, Loeb and Norton, PSEBM, 101, 866(1959)). ATP is absent if the homogenate is preincubated with RNAse but a level of ATP is maintained if the RNAse preincubation is carried out in the presence of an autoclaved extract of fresh liver. The active component of liver extract is not retained by a Dowex-1 (Cl) column but is separable from other unrelated material in the Dowex-1 filtrate by column electrophoresis ('Geon'resin, 0.02 M pH 7.0 phosphate buffer) and precipitation with sodium chloride and ethanol. Active material obtained in this way is RNA with a base distribution characteristic of liver (A=U=10, C=31, G=42). Since the factor as encountered in autoclaved liver extract is not retained on an anion exchange resin but migrates as an acid on electrophoresis, it is suggested that the factor from this source is a nucleoprotein. The material appears to function either as a "cofactor" in ATP formation or as an "inhibitor" of ATPase activity.

12 **Biochemistry**

PHOSPHORYLATION COUPLED TO ELECTRON TRANSPORT INITIATED BY QUINONES. Earl E. Jacobs*, Dartmouth Med. School, Hanover, N.H., and F.L.Crane, Univ. of Texas, Austin, Texas.

Phosphorylation coupled to electron transport initiated in whole rat liver mitochondria by catalytic amounts of silicomolybdate, ferrocyanide and cytochrome c is characteristically less stable than that coupled to succinate oxidation; the instability may be attributed to loss or modification of some structural or chemical factor induced by this manner of initiating electron transport (Jacobs and Sanadi, B.B.A. 37, 1960). The oxidation of exogenous DPNH andascorbate can also be mediated by catalytic amounts of various quinones. Electron transport initiated by benzoquinones (Coenzymes Q_0, Q_1, Q_2, duroquinone) is antimycin-sensitive while that initiated by most napthoquinones is antimycin-insensitive. In either case the related P/O ratios are less than one and approach zero as the quinone concentration is increased. The low P/O ratios observed with the higher concentrations of quinones may be due to their demonstrated uncoupling action at these concentrations. However, in order to explain the low P/O ratios obtained with low concentrations of quinones, it may be necessary to postulate the existence of an exogenous electron donor (quinone) — endogenous electron acceptor complex which subsequently prevents a phosphorylation normally associated with the endogenous electron acceptor.

13 **Biochemistry**

DIRECT SPECTROPHOTOMETRIC ASSAYS OF UBIQUINONE-COENZYME Q IN MITOCHONDRIA. Britton Chance, Johnson Foundation, University of Pennsylvania, Philadelphia, Pennsylvania.

Addition of succinate to glutamate-treated guinea-pig kidney mitochondria causes absorbancy decreases in the region 255-300 mμ and the difference spectrum has a shoulder at 275 mμ. A part of this absorbancy change is due to succinate-linked DPN reduction (Chance and Hollunger, Fed. Proc. 16:163, 1957) but at 275 mμ the reduction of quinone (UQ) contributes considerably, in agreement with spectrophotometric assays of DPN-depleted mitochondria or solvent extraction of intact mitochondria (Chance and Redfearn, in preparation) which give 1.3-1.6 x 10^{-9} moles UQ/mg protein. Spectrophotometric assays indicate ratios of DPNH, UQ, and flavin relative to cytochrome c to be 14, 4, 4 to 1. Some reactions which do not alter the oxidation level of DPNH show changes at 275mμ; succinate addition to glutamate and Amytal-treated mitochondria causes UQ reduction, but does not if Antimycin-A is used instead of Amytal. Phosphate (6mM) does not measureably alter the absorption at 275-300 mμ (but cf Hatefi et al. Biochem Biophys Acta. 31:490-512, 1959). The kinetics of UQ reduction when corrected for concommitant DPN reduction are similar in rate, and are slower than those in DPN-depleted mitochondria, suggesting that DPN acts as an oxidant for UQ in succinate-linked DPN reduction. Dr. Takeru Ito collaborated in these experiments.

14 **Biochemistry**

ADENINE NUCLEOTIDE EXCHANGE PROPERTIES OF MYOKINASE. B.C. Pressman. VA Hosp. and Univ. of Kansas, Kansas City, Mo.

On the basis of the equation of 2 ADP⇌AMP + ATP, myokinase would be expected to transfer P^{32} from α-P^{32} labeled ADP to AMP and ATP at equal rates, when added to a system containing unlabeled AMP, ADP and ATP at equilibrium concentrations. In such a system whole rat liver mitochondria promote AMP/ATP labeling ratios exceeding 2. A partially purified enzyme possessing myokinase activity and giving AMP/ATP label ratios of 6 has been obtained from buffer extracts of mitochondrial acetone powders. The buffer residue, upon further extraction with Triton X-100, yields a fraction with AMP/ATP ratios of 0.3. Although the low AMP/ATP labeling ratio of the Triton extracts could be due in part to enzymes catalyzing direct isotopic exchange between ADP^{32} and ATP, the possibility that the myokinase activity of this fraction is due to contamination by the buffer-extractable myokinase can be ruled out. The likelihood that the exchange and myokinase activities of each fraction are due to one enzyme is increased by the fact that even highly purified, recrystalized, rabbit muscle myokinase gives AMP/ATP labeling ratios other than unity (ca. 1.4). The implications of the exchange reactions on the mechanisms of these two liver myokinases and their relationship to other phosphate transferring enzymes will be discussed. (Supported by a grant from NIH.)

15 **Biochemistry**

CALORIMETRIC MEASUREMENTS DURING BIOLOGICAL OXIDATIONS. Ronald W. Estabrook, and Herbert Gutfreund*, Johnson Foundation, University of Pennsylvania, Philadelphia, Pennsylvania.

In order to evaluate and establish thermodynamic constants for processes associated with biological oxidations and oxidative phosphorylation, a calorimeter capable of determining the small changes in temperature occurring during oxidative reactions has been constructed. Incorporation of an oxygen electrode into the calorimeter permits the simultaneous monitoring of the steady state rate of oxygen uptake. Studies with the succinoxidase and DPNH oxidase system of a Keilin and Hartree heart muscle preparation give values of ΔH oxidation equal to 35 and 54 kcal/mole for succinate and DPNH respectively. Experiments with tightly coupled mitochondria prepared from rat liver tested under conditions of optimal oxidative phosphorylation in the presence of ADP, and oxidation in an uncoupled state such as in the presence of DNP, show preliminary values for heat production of 25 kcal/mole with ADP as acceptor and 35 kcal/mole in the presence of 5 x 10^{-5} m DNP.

16 **Biochemistry**

STRUCTURAL CHANGES LINKED TO OXIDATIVE PHOSPHORYLATION IN MITOCHONDRIAL MEMBRANES. Lester Packer (intr. by J. H. Copenhaver.) Dartmouth Med. School, Hanover, N. H., and the Univ. Texas Southwestern Med. School, Dallas, Texas.

Studies on the swelling-shrinking phenomena in mitochondria led to an examination of light-scattering changes in mitochondrial membrane fragments (Packer and Tappel, J. Biol. Chem. 235 No. 1, 1960). Scattering increases were dependent on electron transport coupled to phosphorylation, adenine nucleotides, and Mg or Mn ions. Reversal of scattering is catalyzed by oxidation of the respiratory chain, phosphate or arsenate, and uncoupling agents. Scattering changes in mitochondria with these reagents are accompanied by volume changes, but no evidence for volume changes has been obtained in fragments. Physical studies were undertaken to clarify the nature of the change in fragments of which scattering is a measure. Scattering increases are accompanied by a decline in viscosity; reversal of scattering, as with uncoupling agents, increases viscosity. Examination of the full angular dependence of scattering indicated a change to a more compact structure in the state of increased scattering. The fragments yield linear Zimm plots which extrapolate to the same intercept. It was concluded that the structural change occurring in membrane fragments leads to a change in shape or symmetry and that the minimal molecular wt. for a phosphorylating unit in these preparations exceeds 10^6.

17 **Biochemistry**

STIMULATION OF ATP HYDROLYSIS BY ARSENATE IN PHOSPHORYLATING PARTICLES. Charles L. Wadkins (Intr. by A. L. Lehninger), Johns Hopkins Medical School, Balto., Md.

The mechanism whereby arsenate uncouples oxidative phosphorylation has been interpreted as a competition with inorganic phosphate in a reaction with a high energy intermediate formed during respiration. Evidence that arsenate also induces the hydrolysis of such an intermediate has been obtained. In a tightly coupled system arsenate stimulates respiration in the absence of inorganic phosphate and of phosphate acceptor. In addition, arsenate stimulates the hydrolysis of ATP by intact mitochondria and also by phosphorylating submitochondrial fragments prepared with digitonin. This reaction was measured by using ATP labeled at the terminal phosphate with P^{32} and determining the radioactivity of the inorganic phosphate formed. ATP hydrolysis is greatly stimulated by arsenate, but not by other anions such as sulfate, borate, phosphate or nitrate. Maximum stimulation by arsenate occurs at pH 7.5 and requires Mg^{++}. The arsenate stimulated ATP-ase is inhibited by azide and phenylmercuric acetate at concentrations which also inhibit the DNP stimulated ATP-ase. It appears possible that arsenate may form an arsenylated analog of a phosphorylated intermediate of sufficient instability as to undergo spontaneous decomposition and thus accelerate ATP-hydrolysis.

18 **Biochemistry**

AN UNCOUPLER OF OXIDATIVE PHOSPHORYLATION FROM LEUCOCYTE MITOCHONDRIA EXHIBITING BACTERICIDAL ACTIVITY. **M. Fishman* and M.E. Pullman. The Public Health Research Institute of The City of New York, Inc., New York, New York.**

Bactericidal activity has been demonstrated in extracts of mitochondria isolated from rat polymorphonuclear leucocytes. The responsible agent was tentatively identified as a lipoprotein (J. Exp. Med. 105, 521, 528, 1957). The molecular weight of the bactericidal substance was found to be 1 x 10^6 as determined by sedimentation rate. The bactericidal activity was reversed by serum albumin, and to a lesser degree by beta lactoglobulin, whereas serum gamma globulin was without effect. The effect of these three proteins on bactericidal activity is similar to that observed on the activity of an uncoupler of oxidative phosphorylation isolated from rat liver mitochondria, (Science, 123, 1109, 1956). In view of these similarities the effect of the bactericidal agent on oxidative phosphorylation was investigated. Addition of 0.2-0.3 γ nitrogen of the purified bactericidal agent to rat liver mitochondria resulted in 80 to 90% uncoupling of oxidative phosphorylation. At higher concentrations respiration was inhibited. As with the bactericidal activity, the uncoupling of oxidative phosphorylation was reversed by serum albumin and was not inactivated by either heat or dialysis.

XUM

19 *Biochemistry*

EVIDENCE FOR DITHIOL FUNCTION IN OXIDATIVE PHOSPHORYLATION BASED ON UNCOUPLING BY ARSENITE. A. L. Fluharty* and D. R. Sanadi. Gerontology Branch, National Heart Institute, and Baltimore City Hospitals, Baltimore 24, Maryland.

The possible participation of vicinal dithiols in oxidative phosphorylation (J. Biol. Chem., 223, 147, 1956) has been examined with the use of arsenite, a more specific reagent for dithiols. The oxidation of glutamate by rat liver mitochondria is inhibited by 0.05 mM arsenite while β(OH) butyrate oxidation is affected only at 0.5 mM. The oxidation of succinate is stimulated by arsenite, particularly at high levels (3 mM), with simultaneous partial uncoupling. Attempts to reverse the uncoupling by 2,3-dimercaptopropanol (BAL) revealed that much lower levels of arsenite (< 0.1 mM) in combination with BAL completely uncouple phosphorylation with no effect on succinate oxidation. Addition of higher levels of BAL to mitochondria thus uncoupled restores phosphorylation. Monothiols at equivalent levels were ineffective either in potentiating the uncoupling or in the reversal. Low concentrations of arsenite and BAL individually have no effect on ATPase activity; together they induce as active an ATPase as is induced by 2,4-DNP. The effect is again reversible by excess BAL. One possible explanation of the data is that arsenite, perhaps due to its charge, can not readily reach a functional dithiol; crossing of the barrier is enabled by combination with BAL. Arsenite is then transferred to a dithiol involved in phosphorylation. Reversal by excess BAL is determined by mass action.

20 *Biochemistry*

INHIBITION OF DINITROPHENOL-STIMULATED MITOCHONDRIAL RESPIRATION BY INORGANIC PHOSPHATE AND ADP. Rudolf H. Eisenhardt*, and Otto Rosenthal. Harrison Department of Surgical Research, Schools of Medicine, University of Pennsylvania, Phila., Pa.

The rate of O_2 uptake of 2,4-dinitrophenol (DNP) uncoupled rat liver mitochondria in the absence of inorganic phosphate (P_i) and ADP with beta-hydroxybutyrate (BOH) as substrate is decreased by approximately 30% when P_i and ADP are added. The increase in the rate of O_2 uptake upon subsequent addition of succinate to this system is one-half of that observed when P_i and ADP are added before DNP. These effects are absent if glutamate is substituted for BOH. The differences between these two substrates, both DPN-linked, suggest the presence of partially different pathways and a selective action of DNP. O_2 consumption was determined at room temperature in glycylglycine buffer with an O_2 electrode. Borst and Slater (Nature, 184:1396, 1959) recently reported that the manometrically measured O_2 uptake of uncoupled mitochondria incubated with glutamate in a system depleted of P_i by the hexokinase-glucose trap is increased upon addition of P_i. Incorporation of the hexokinase-glucose trap did not alter our results. Preliminary data indicate that these discrepancies may be due to the longer incubation intervals required by the manometric method employed by Borst and Slater. (Supported by grant P-196 of the American Cancer Society).

21 *Pathology*

THE EFFECTS OF GLYCEROL ON OXIDATIVE PHOSPHORYLATION BY SUSPENSIONS OF MITOCHONDRIA AFTER FREEZING. Carmen Privitera, Donald Greiff and Maurice Myers. Marquette Univ. Sch. of Med., Milw., Wis. and St. Louis Univ., St. Louis, Mo. (Intr. by H. Pinkerton).

Data were obtained for oxidative phosphorylation by suspensions of mitochondria frozen slowly at -76° in a 10% glycerol-isoconic sucrose solution and stored at -65° for 15 days. Two preparations were used, 1) glycerol was added to mitochondria suspended in isotonic sucrose (post-preparative), 2) the preparation of mitochondria was carried out completely in a 10% glycerol-isotonic sucrose solution (pre-preparative). The mean values for micromoles of phosphorous esterified and the micro-atoms of oxygen used ± the standard errors of the means are shown below.

	Control		Freeze-Thaw		Stored	
	P	O	P	O	P	O
Sucrose	26.7±.89	9.5±.26	19.7±1.9	8.3±.52	16.1±.53	7.7±.36
Glycerol-Sucrose (post-prep)	26.6±.69	10.1±.23	20.5±1.2	8.7±.40	16.5±1.0	7.8±.60
Glycerol-Sucrose (pre-prep)	27.6±1.2	9.8±.49	26.4±1.1	9.3±.35	24.4±.67	8.4±.21

(Supported by a grant from the Atomic Energy Commission)

22 *Nutrition*

PURIFICATION OF A MYOKINASE FROM HOG LIVER. M. Chiga (intr. by L. T. Samuels). U. Utah Coll. of Med., Salt Lake City, Utah.

The major portion of the activity $ADP^{32} \longleftrightarrow ATP^{32} + AMP$ of the aqueous extract of hog liver is inhibited substantially by AMP plus NaF; but is neither inhibited nor precipitated by $AgNO_3$, $HgCl_2$ or para-hydroxymercuribenzoate. Utilizing these characteristics this nucleoside monophosphate kinase was purified by a combination of the following steps: 1) Metal treatment: $AgNO_3$ 0.009M at 40° for 5 min., or $HgCl_2$ 0.0045M in the cold. 2) Concentration with ammonium sulfate at 0.7 saturation. 3) $AgNO_3$ treatment 0.0009M at 40° for 5 min. 4) Ammonium sulfate, 0.3-0.6 saturation. 5) Acetone 35%-50% fractionation. Further fractionations including treatments with aluminum hydroxide gel, cation exchange resin, etc., result in higher purification. The nucleoside diphosphokinase-free preparation thus obtained is tentatively identified as an AMP-nucleoside triphosphate transphosphorylase. A preparation was obtained which formed 40 μmoles of ATP from ADP per mg. protein per min. at 25° as assayed with a combination of hexokinase and glucose-6-phosphate dehydrogenase.

022. BIOLOGICAL REGULATION

1 *Physiology*

PROPERTIES OF THE CAROTID SINUS MECHANISM. L. H. Peterson, E. Feigl* and P. Gouras.* University of Pennsylvania School of Medicine, Philadelphia, Penna.

Carotid sinus dimensions and pressure and the electrical activity of the receptors have been measured simultaneously, instantaneously and continuously in living, intact, anesthetized dogs. From these studies the mechanical properties (elasticity and viscosity) of the sinus wall have been computed and correlated with wall motion, arterial pressure and receptor activity. It has been found that (i) the sinus wall is the most distensible arterial segment known. This difference is due primarily to the geometry of the sinus rather than to differences in the mechanical properties of the sinus wall, (ii) the "normal" strain (percent change in dimensions) resulting from arterial pulse pressure is 3 to 4%, (iii) the mechanical properties of the sinus wall can change by at least 100% following local application of vasoactive materials indicating active wall properties (probably smooth muscle), (iv) the wall properties appear to change reflexly, (v) the receptor activity changes with respect to intrasinus blood pressure following changes in wall properties. These findings suggest that it is misleading to term this a pressure receptor mechanism. (Supported by grants from the National Heart Institute, U.S.P.H.S. and the Office of Naval Research.)

2 *Biochemistry*

RESPONSE PATTERNS TO ALCOHOL IN BABY CHICKS. Frank L. Siegel* and Roger J. Williams. Clayton Foundation Biochemical Institute and Dept. of Chemistry, University of Texas, Austin.

In pursuance of the biochemical individuality approach to alcoholism a coordinated study involving a total of 46 baby chicks has demonstrated that each chick exhibits a distinctive over-all pattern of responses to alcohol. This pattern is composed of (1) voluntary alcohol consumption; (2) appetite impairment following administration of alcohol; (3) susceptibility to alcoholic intoxication; (4) rate of alcohol metabolism; (5) retention of alcohol in the liver; (6) retention of alcohol in the brain. The latter three items involved tracer studies on 11 of the 46 chicks. Individual chicks also exhibit distinctive organ weight patterns and patterns of blood morphology. These studies throw light on the problem of human alcoholism.

3 *Biochemistry*

A STUDY OF THE RELATION OF ASYMMETRICAL DIMETHYLGUANIDINE TO TOXEMIA OF PREGNANCY. M.X. SULLIVAN, Georgetown University, Washington, D.C.

In a number of cases of toxemia of pregnancy we obtained evidence to indicate that diamines occur in the urine. Since Zeller (Helv.Chim. Acta 21,1645, 1938) found that diamine oxidases are inhibited by asymmetrical dimethylguanidine which could arise from creatine by decarboxylation we developed a highly differential test for asymmetrical dimethylguanidine sensitive to 0.1 mg per ml. The test is made as follows: to 0.5 mg of the guanidine on 0.1N HCl are added 0.1 ml of a 1% solution of acetyl benzoyl in 50% alcohol and 1 ml of 1N NaOH. After gentle shaking to mix and letting the solution stand for 10 minutes at 20-25°C the mixture is extracted with 7 ml toluene. Only asymmetrical dimethylguanidine gives a violet layers. No evidence has been obtained that asymmetrical dimethylguanidine occurs in the urine of toxemia of pregnancy cases. However, the guanidine is a very reactive compound and ties with many other substances, so now we are striving to untie it without loss and to make the test much more sensitive. (Supported by a grant from the National Heart Institute)

4 *Biochemistry*

GLYCOLYSIS AS A REGULATOR OF FATTY ACID AND CHOLESTEROL SYNTHESIS. E.C. Layne (Instr. by M Andersch) Univ. of Md. Sch. of Med., Balto., Maryland.

We have previously proposed (Fed. Proc. 18, 88, 1959) that the differential as well as the net synthesis of both fatty acid and cholesterol is regulated by the total level of reduced pyridine nucleotides. As suggestive evidence for such a mechanism we have reported that the differential incorporation of acetate-C^{14} into a crude extract of fatty acid and into digitonin-precipitable material is sensitive to the level of added glucose-6-phosphate. In the present report evidence is presented to indicate that our previous methodological assumptions are correct. The radioactivity of purified cholesterol isolated from the mixed digitonides is proportional to the crude activity. When fatty acids are isolated from the crude extract by countercurrent distribution, net incorporation is proportional to the level of glucose-6-phosphate added, whereas distribution is unchanged. The rate of generation of reduced pyridine nucleotides is proportional to the level of glucose-6-phosphate added.

5 *Biochemistry*

CHANGES IN PYRUVATE METABOLISM IN THE TCA CYCLE IN POSTNATAL DEVELOPMENT. A.D. Freedman, Columbia U. and Andrew Nemeth, U. of Penn. (intr. by S. Graff).

Pyruvate entering the TCA Cycle by decarboxylation to acetyl CoA can be differentiated from pyruvate entering as a dicarboxylic acid, (Freedman, A.D., and Graff, S., J. Biol. Chem., 233, 292 (1958)) by degradation of glutamic acid after administration of DL-alanine-$2C^{14}$. This study was performed on the livers of guinea pigs 4 hours, 10 hours, 10 days and 30 days old. The percentage of pyruvate entering the TCA cycle as acetyl CoA declines between 4 and 30 hours, then increases to the initial values at 10 days, and at 30 days is 3 times initial values. The initial decline can be correlated with decreasing liver fat and glycogen and increasing fat oxidation. The rising curve is correlated with increasing liver TPNH. The branch point pyruvate responds to, and is part of, the developmental sequence in early postnatal life. This work was supported by U.S.P.H.S. Grant C-3141.

6 *Biochemistry*

IN VIVO TURNOVER OF LIVER PYRIDINE NUCLEOTIDES. V. Stollar* (intr. by P. L. Munson.) Grad. Dept. of Biochemistry, Brandeis Univ., Waltham, Mass.

The relative turnover of the different pyridine nucleotides has been studied by injecting either radioactive $P^{32}O_4$, C^{14} uniformly-labeled glucose, or C^{14}-nicotinic acid. The animals were sacrificed after one hour and the pyridine nucleotides isolated. The reduced coenzymes were oxidized with phenazine methosulfate. After injection of P^{32} it was found that the specific activities of the phosphates in DPN and DPNH were similar. The TPN obtained from the TPNH was also found to be highly labeled, but most of the radioactivity was found in the monoester phosphate grouping. The pyrophosphates of TPN had a very low specific activity as compared to the phosphates of DPN. C^{14}-labeled glucose was incorporated into DPN with 8 times the specific activity as that of TPN. This work suggests that the DPN moiety of TPN is not in rapid equilibrium with DPN and this appears to be of interest because of the high turnover of the monoester phosphate grouping of TPN. Studies with C^{14}-labeled nicotinic acid gave equal specific activities with DPN, DPNH, TPN and TPNH. The fact that the DPN and TPN forms show similar specific activities with regard to the nicotinamide portions, but not with regard to the ribose and pyrophosphate moieties indicates that the exchange reaction involving nicotinamide occurs in vivo.

7 *Biochemistry*

THE ENZYMATIC REDUCTION OF PYRIDINE HYDROXAMIC ACIDS TO AMIDES BY MOUSE LIVER MITOCHONDRIA. P. F. Hirsch* (intr. by H. P. Klein.) Grad. Dept. of Biochemistry, Brandeis Univ., Waltham, Mass.

An enzymatic reduction which stoichiometrically converts nicotinyl hydroxamic acid to nicotinamide has been locallized in mouse liver mitochondria. The mitochondrial preparation will also catalyze 1) the conversion of N^1-methyl nicotinyl hydroxamic acid to N^1-methyl nicotinamide, and 2) the hydroxamic acid analogue of DPN to DPN. The conversion of these pyridine hydroxamic acids into amides requires the addition of either DPN, DPNH, or the acetyl pyridine analogue of DPN. The reaction is further enhanced by Mg^{++}, sodium glutamate, and anaerobic incubation. This enzymatic system is different from a mitochondrial enzyme which causes the hydrolysis of octanoyl hydroxamic acid, but does not require DPN for its action. The conversion of the pyridine hydroxamic acid to the amide appears to proceed by a reduction of the hydroxamic acid group rather than an exchange reaction. A possible physiological role for the enzyme in DPN synthesis will be presented.

8 *Biochemistry*

CINNAMYL AND COUMARYL ESTER INTERMEDIATES IN THE SYNTHESIS OF CHLOROGENIC ACID. CARL C. LEVY AND MILTON ZUCKER (intr. by I. Zelitch) Conn. Agr. Exp. Station, New Haven, Conn.

When disks of potato tuber tissue are cultured in .05 M L-phenylalanine or .005 M trans-cinnamic acid, the net synthesis of chlorogenic acid (3,4-dihydroxycinnamyl-quinic acid) is stimulated several fold. Paper chromatography of the extracts of these tissues has revealed the presence of two esters not found in control cultures. These compounds have been tentatively identified as cinnamyl-quinic and 4-hydroxycinnamyl-quinic (p-coumaryl-quinic) acids, the former accumulating in trace amounts only. Culture of disks in .002 M p-coumaryl-quinic acid stimulates the synthesis of chlorogenic acid. Moreover, this ester can be converted into chlorogenic acid by cell free extracts of potato in the presence of ascorbic acid. The enzymatic conversion is inhibited by phenylthiourea and 4-chlororesorcinol, suggesting that polyphenoloxidase is involved. Experiments with randomly labeled L-phenylalanine-C^{14} also show a close relationship between the mono- and dihydroxycinnamyl esters. The data reported suggest a pathway of synthesis of chlorogenic acid involving cinnamyl and p-coumaryl-quinic acids as intermediates. (Supported in part by a grant from the National Science Foundation.)

XUM

9 *Biochemistry*

THE INDUCTION OF TWO SIMILAR ENZYMES BY ONE INDUCER. A TEST CASE FOR SHARED GENETIC INFORMATION. Marshall W. Nirenberg (intr. W. B. Jakoby.) Natl. Insts. of Health, Bethesda, Md.

This investigation asks whether a portion of one gene contains information for the synthesis of a protein subunit which might be an integral part of two or more enzymes. A strain of Pseudomonas fluorescens formed an inducible γ-hydroxybutyric acid dehydrogenase (Reaction 1) when grown upon γ-hydroxybutyric acid (γ-HBA), and an inducible β-hydroxypropionic acid dehydrogenase (Reaction 2) when grown upon β-hydroxypropionic acid (β-HPA) Nirenberg, M. W. and Jakoby, W. B., J. Biol. Chem. (in press.)

1) γ-HBA + DPN $\rightleftharpoons$ Succinic Semialdehyde + DPNH + H^+
2) β-HPA + DPN $\rightleftharpoons$ Malonic Semialdehyde + DPNH + H^+

An attempt was made to induce the reversible γ-HBA dehydrogenase by both the reactant and the product of the reaction; only γ-HBA was effective. A series of mutant strains blocked in reaction 1 were obtained. Strikingly high β-HPA dehydrogenase levels were found in these strains when γ-HBA was added. Analysis demonstrated that γ-HBA at low concentrations induced the formation of γ-HBA dehydrogenase, and, at higher concentrations, induced the formation of both γ-HBA and β-HPA dehydrogenases. Genetic information did not appear to be shared; instead γ-HBA was found to serve as an inducer for two similar enzymes in different metabolic pathways.

10 *Biochemistry*

LACK OF REGULATORY ACTION OF CEREBROSPINAL FLUID ON BLOOD GLUCOSE CONCENTRATION. Henry A. Sloviter. Dept. Neurosurgery and Harrison Dept.Surg.Research, Univ.of Penna.,Philadelphia.

It had been reported that an increase in the glucose concentration of the cerebrospinal fluid (CSF), produced by intrathecal injection of glucose solution, caused a definite hypoglycemia (Proc.Soc.Exp.Biol.& Med.,65:23,1947). In the present experiments, solutions of glucose and other substances were injected into the cisterna magna of dogs which were, in most cases, under pentobarbital anesthesia. Blood samples were analyzed for glucose by a glucose oxidase method. It was found that neither a small nor a large increase in CSF glucose concentration caused any significant change in blood glucose concentration. A decrease in CSF glucose concentration produced by replacing a large part of the CSF by saline solution also had no significant effect on blood glucose concentration. Injection of insulin into the CSF produced a hypoglycemic response only after considerable delay and was probably due to the effect of insulin which was absorbed from the CSF into the blood. Ribose, which causes hypoglycemia when given intravenously, and 2-deoxy-D-glucose, which causes hyperglycemia when given intravenously, were also without effect when injected into the CSF. Similar results were obtained in two cases where the injections of glucose into the CSF were made in unanesthetized dogs. (Aided by U.S.P.H.S. Grant B-2032)

11 *Biochemistry*

METABOLIC PATHWAYS ASSOCIATED WITH PHAGOCYTOSIS. W. Evans* and M. L. Karnovsky. Harvard Medical School, Boston, Massachusetts.

Previous work established a number of changes in metabolism of polymorphonuclear leucocytes when inert particles are phagocytized (J. Biol. Chem. 234, 1355 (1959)). Experiments have now been performed to provide information concerning the mechanism(s) underlying the metabolic concomitants of phagocytosis. CO_2 fixation by these cells is considerable and lactate isolated from cells incubated in the presence of $C^{14}O_2$ was labeled only in the carboxyl carbon. Glycogen-glucose carbon-six contained about 15% of the activity when glucose-1-C^{14} was used as substrate, whereas no randomization was observed with glucose-6-C^{14}. Methylene blue stimulated oxygen uptake three-fold, and this substance or pyruvate stimulated conversion of glucose carbon-one to CO_2. In a survey of cyanide insensitive pathways, using intact cells, homogenates or subcellular fractions, the large granules of these cells were found able to oxidize DPNH or TPNH. The rate of oxygen uptake with the former substrate was twice that with the latter. The addition of the supernatant fraction inhibited this activity of the granules. This cyapide insensitive oxidation of reduced pyridine nucleotides may be relevant to the cyanide insensitive stimulation of oxygen uptake and appearance of glucose carbon-one as CO_2 during phagocytosis.

12 *Pharmacology*

THE EFFECT OF COLCHICINE ON TAURINE EXCRETION. J. J. Kocsis and V. J. Kostos.* Dept. of Pharmacology, Jefferson Medical College, Philadelphia 7, Pa.

Many of the characteristic biological effects of colchicine parallel those produced by X-irradiation. Since X-irradiation increases the urinary excretion of taurine (Kay and Entenman, Fed. Proc. 13: 520, 1954), we studied the effect of colchicine on taurine excretion. Taurine excretion in the urine of 12 fasted rats during two 24 hour control periods prior to treatment averaged 2 micromoles/hr., whereas in the 14 hour period immediately following colchicine in doses up to 3.2 mg/kg the excretion of taurine was more than 5 x the control values. However, with higher doses of colchicine (6.4 mg/kg) taurine excretion was only 2 x the control level. The fact that no changes were observed in the taurine concentration of the small intestine, brain or heart at 3, 6, 12 and 24 hours after the administration of colchicine (1.6 mg/kg) suggests that preformed organ taurine may not be the source of the extra taurine excreted in the urine following treatment. (Supported by NIH research grant CY-3349)

13 *Pharmacology*

EFFECTS OF 17-ETHYL-19-NORTESTOSTERONE ON HYDROCORTISONE-INDUCED CHANGES OF RAT BONE. Joseph Grossman, Howard Schwartz, Joseph Samachson and Herta Spencer (intr. by Philip Lief) Montefiore Hospital, New York.

The protein-anabolic effect of 17-ethyl-19-nortestosterone, Nilevar, was investigated in mature, Wistar stock, albino rats receiving corticosteroids. The rats received a known amount of diet, aliquots of which were analyzed for Ca, P and N. Four groups of 9 rats each were studied: Group A (control) received 0.5 ml water/day subcut., Group B, 100 mg hydrocortisone/kg/day Group C, 25 mg Nilevar/day, i.m. and Group D, the combination of hydrocortisone and Nilevar. The Ca, P and N content of both humeri of each rat was determined at the end of the study. The nitrogen content of bone of Group B, receiving corticosteroids, was significantly less than that of the controls, while that of Group D, receiving corticosteroids and Nilevar, was significantly greater than that of Group B but similar to the control group. The average phosphorus concentration of bone of all groups was similar. The bone calcium concentration of Group A was slightly, but not significantly, higher than that of Group B and similar to Group D. The data suggest that 17-ethyl-19-nortestosterone protects against the protein-catabolic effect of hydrocortisone on osseous tissue. (Supported by a grant from the U.S. Public Health Service.)

.023. BIOLOGICAL REGULATION— Body Temperature

1 *Physiology*

The Cutaneous Vascular and Sweating Responses of the Forearm. Leo C. Senay, Jr.* and Alrick B. Hertzman, St. Louis University School of Medicine, St. Louis, Mo.

When the nude subject was exposed to a slowly rising ambient temperature (from 25° C. to 45° C. in 3 hours), the cutaneous forearm blood vessels dilated in a consistent and almost predictable manner. Whether a rapid (from 30° C. to 50° C. in 20 min.) or slowly rising ambient temperature was utilized, this vasodilatation was curvilinearly related to the forearm skin temperature. With the rapid rise in ambient temperature, the onset of sweating appeared to be related to the point of inflection in these curves; i.e., where the rate of dilation markedly increased. This was not true with the slower rises in ambient temperature. The occurrence of and increase in forearm sweating was unrelated to the progress of the vasodilatation. When the ambient temperature was rapidly cycled (from 25° C. to 45° C. to 25° C. in one hour) large increases in local skin temperature and forearm vasodilatation occurred without detectable sweating. An imposed temperature of 30° C. by a thermode on a small area of the forearm depressed or prevented the occurrence of vasodilatation, even though sweating took place under the thermode. (Supported by Contract AF 33(616)-3357 and grant H-3489, USPHS.

XUM

2 *Physiology*

BEHAVIOR IN THE COLD AFTER ACCLIMATIZATION. Victor G. Laties and Bernard Weiss (intr. by E. K. Marshall, Jr.) Johns Hopkins Univ. School of Medicine, Baltimore, Md.

Sixteen male, experimentally naive, albino rats were each given one 16-hour session in a heat reinforcement chamber (Am.J.Physiol.,197:1028,1959) located in a room maintained at 2°C. While in this chamber, the rat could, by pressing a lever, get 2-sec. bursts of heat from a 250-watt infrared lamp located above him. Half the animals had been living at 2°C for from 30 to 41 days before their experimental sessions. The rest had been living at 25°C. All had had food and water ad libitum. Eight hours before an animal from the acclimatized group started an experimental session, it was removed from the cold room. Body hair was removed with an electric clipper shortly before a session began. Results: Acclimatized rats generally waited longer than control rats before starting to work at a steady rate for heat. A Wilcoxon Unpaired Replicates Test showed that the difference was significant at the .01 level. (Supported by a grant from the U.S. Public Health Service, NINDB (B-865)).

3 *Physiology*

RELATIONSHIP OF CHANGING LEVELS OF PHYSICAL FITNESS TO HUMAN COLD ACCLIMATIZATION. E. J. Heberling and T. Adams (intr. by L. D. Carlson) Arctic Aeromedical Lab. and Dept. of Physiology & Biophysics, Univ. of Washington School of Med., Seattle, Washington.

Five adult male Caucasians were exposed nude in a cold chamber (10° C) for 1 hour before and after a physical training program, and following a six-week winter (Jan.-Feb.) field bivouac in interior Alaska. Body temperatures during the acute cold exposure were the criteria by which the effects of the changing levels of physical training and of the "cold-acclimatizing" field encampment were judged. After the physical training program (before bivouac) the skin and extremity temperatures were significantly higher than pre-training measurements, but no differences in these values were seen after the field bivouac when physical training levels remained unchanged and the variable of cold exposure operated alone. These data confirm an earlier suggestion that these indices of "acclimatization" may appear as a result of chronically elevated activity levels. Dietary measurements showed equal caloric intakes in the training and bivouac phases, although formal activity requirements were discontinued in the latter test period. These data open for question the interpretation of physiological changes observed after winter bivouac or field exposures as being "cold induced."

4 *Physiology*

THE CONTROL OF THERMOREGULATORY RESPONSES IN THE DOG AFTER ALTERATION OF PERIPHERAL SENSITIVITY. R.H. Helmendach and J.P. Meehan (intr. by J.E. Thomas) Coll. Med. Evang., Loma Linda, Calif. and Univ. Southern Calif., Los Angeles, Calif.

Peripheral and central thermosensitive areas are known to be present in mammals. The relative importance of these areas in the initiation and suppression of thermoregulatory mechanisms is not well delineated. Many studies have been performed involving stimulation or ablation of thermoregulatory centers. The present studies were performed to give information concerning peripheral thermosensitivity. In order to examine thermoregulatory effects following peripheral thermosensitivity changes, topical agents were applied to the body surfaces of unanesthetized dogs which were then subjected to heat and cold stresses. The effects of these topically applied agents on the initiation and suppression of shivering and panting were noted. Upon application of menthol, both shivering and panting occurred sooner than in controls. Conversely after application of a topical anesthetic, Tronothane hydrochloride, shivering and panting occurred later than in control animals. In most instances colonic temperatures were unchanged or paradoxically changed when panting or shivering were initiated or suppressed. The results show that peripheral sensitivity was important in the control of the thermoregulatory responses observed in these studies.

5 *Physiology*

RESPIRATORY EFFORT AND SHIVERING. R. W. Bullard*(Intro. by C. A. Bunde.) Dept. Physiol., Ind. Univ. School of Med. Indianapolis, Indiana.

Nude men exposed to 6°C. air, voluntarily hyperventilated for 10-minute periods either a cold dry air (6°C., 50% r.h.) or a warm moist air (37°C., 100% r.h.). Shivering was assessed by recording electromyographic and mechanical activity and by visual observation. At the start of the hyperventilation period, shivering was inhibited for 2 to 4 minutes.All rates of hyperventilation of cold dry air then markedly increased shivering. Moderate hyperventilation (20 to 30 l/min) of warm moist air likewise increased shivering over that of control cold exposure periods. Greater hyperventilation of the warm air generally inhibited shivering. Because similar results were obtained when subjects hyperventilated air with 2½% carbon dioxide, the effects are not due to hypocapnia. Moderate respiratory effort per se increased shivering activity, but severe hyperventilation, in which breathing effort was a large proportion of the total metabolism, inhibited shivering. With cold air hyperventilation, this inhibition was overcome either by increased respiratory heat loss or by activation of thermal receptors in the respiratory passages. (Supported by Contract DA-49-007-MD-974, Office of the Surgeon General, U. S. Army.)

6 *Physiology*

EFFECT OF 2,4-DICHLOROPHENOXYACETIC ACID ON THERMOREGULATION IN RATS. Frederick N. Sudak and C. Lloyd Claff (intr. by H. L. Barnett) Albert Einstein College of Medicine, New York.

In a previous study the authors have shown that rats treated with 2,4-dichlorophenoxyacetic acid (2,4-D) were unable to maintain body temperatures when exposed to cold. The present study concerns effects of 2,4-D on thermoregulatory mechanisms. Control animals maintained their core temperature constant during a 90 minute exposure to 2.0 + 0.5°C. Core-to-skin thermal gradients increased from 1.3 + 0.2°C. to 2.8 + 0.4°C. after 30 minutes and remained unchanged for the next 60 minutes. No significant change in thermal conductance of skin occurred upon exposure to cold for a 90 minute period in animals treated with 2,4-D. A concomitant decrease in colonic and skin temperature occurred in a linear manner (0.18°C./min.) in this group. Muscle potentials recorded in control and 2,4-D-treated rats under chloralose anesthesia showed that shivering was inhibited in the latter. Calorigenic studies made on cold acclimatized rats indicated that 2,4-D did not interfere with increased oxygen consumption associated with chemical thermoregulation, and therefore may not act at the tissue level in affecting thermogenesis. Indications are that 2,4-D affects thermoregulation by the inhibition of shivering and by increasing heat loss. (Supported by grants H-3563 and H-3836 from the Public Health Service.)

7 *Physiology*

DIET AND BODY TEMPERATURE IN THE COLD, M. B. Kreider*, E. R. Buskirk, and P. F. Iampietro, Army QM R&E Center, Natick, Mass.

In a previous study it was shown that a meal eaten just before retiring decreased the rate of body cooling while sleeping in a cold environment. The present study was undertaken to determine whether the composition of the pre-retiring meal had any significance. Thirteen young soldiers were divided into two groups and fed complete liquid diets of very similar caloric content but of different composition. One group was fed a normal, high fat, high CHO and normal diet on the first, second, third and fourth weeks, respectively, while the second group was on a normal, high CHO, high fat and normal diet during the same periods. Three meals were eaten at 8 AM., 5 PM., and 10:15 PM. at which time 30, 30, and 40 per cent of the total daily calories were consumed, respectively. Measurements of skin (11 points) and rectal temperatures were made at one-half hour intervals throughout the night when the subjects were in sleeping bags at ambient temperatures of -30°F. Protection against the cold was designed to be somewhat inadequate to maintain thermal comfort. Composition of the diet had no effect on rectal, mean weighted skin or toe temperatures throughout the night with the exception that 2 AM., T_R on the high fat diet was lower than on the other diets and at 5 AM. toe temperature was lower on the normal diet than on the other diets. No physiological significance is ascribed to these differences since they were very small.

XUM

8 *Physiology*

CONTINUOUS MEASUREMENT OF METABOLIC RATE IN HUMAN SUBJECTS EXPOSED TO COLD 10°C. R. H. Thompson*, E. R. Buskirk, R. Moore*, and G. D. Whedon. National Institute of Arthritis and Metabolic Diseases, NIH, Bethesda, Md.

Various methods applied in the past to the measurement of metabolic rate in the cold have been based on interval sampling of expired gas concentrations. Although the discrete sampling approach has established sample to sample differences in metabolic rate, the moment to moment changes have not been identified. By using methods for continuous gas analysis and instrumentation described earlier (Fed. Proc. 16:137, 1957), it has been possible to delineate previously obscured features of the metabolic response (oxygen consumption) to cold. Young subjects varying in body composition, clothed only in shorts, were individually exposed for approximately three hours on two or more occasions to 26.6°C (control environment) or 10°C air. Marked inter-individual differences were observed in the metabolic response of these subjects to cold, both in the delay before initiation and the magnitude attained. Characteristic features of the metabolic response were its cyclic nature associated with intermittent shivering and an underlying general increase in oxygen consumption for approximately two hours following its onset. Cyclic changes in oxygen consumption were not reflected by changes in any of the measured body temperatures.

9 *Physiology*

EFFECTS OF FEEDING ON ACTIVITY AND TEMPERATURE IN MICE. W. Parson, W. K. Smith and G. Hollifield (intr. by G. C. Pitts) University of Virginia School of Medicine, Charlottesville, Virginia.

Postprandial body temperature rise has been postulated to produce satiety; and increased running activity during starvation has been thought to be an attempt to maintain temperature. We have studied rectal temperatures, spontaneous running and "jiggle cage" activity in mice during ad lib feeding, starvation and refeeding. In cages permitting spontaneous running activity, mice fed ad lib ran and ate more during the night and their temperatures declined despite marked increases in running activity. Small refeedings after starvation resulted in sharp declines in running activity while the temperatures fell. In small "jiggle cages" mice fed ad lib were more active and ate more during the 12 hours of darkness but their temperatures were higher at the end of this period. Temperatures fell during food deprivation but activity did not increase. Running appears to be a special type of activity related to feeding but unrelated to body control.

10 *Physiology*

CESSATION OF SHIVERING FOLLOWING FOREBRAIN LESIONS IN ANESTHETIZED CATS. F. H. Jacobson, P. H. Craig* and R. D. Squires. Av. Med. Accel. Lab., Naval Air Development Center, Johnsville, Pa.

Shivering ceased after electrolytic lesions were made in the forebrain of 14 cats lightly anesthetized with pentobarbital sodium. Ten of these were further tested for tremor during warming and recooling after the cautery. Shivering resumed in one animal and was stopped by enlargement of the lesion. Cooling rates could be compared in five cats, which cooled faster after operation. Overlapping point lesions were made until shivering stopped. Unilateral lesions sufficed if the electrode was placed within ½ mm of the mid-line. Centers of the lesions ranged from 1 mm rostral to 1 mm caudal to the anterior commissure. Some lesions extended from the corpus callosum to the optic chiasma. Others barely encroached upon the septum pellucidum and continued only into the upper third of the preoptic region. A volume common to all lesions extended approximately ½ mm from the mid-line on one side and divided the anterior commissure and upper third of the preoptic region. Large unilateral lesions 2 mm rostral or ½ mm lateral to this overlap volume were ineffective, as were smaller lesions ½ mm dorsal to it. Shivering was recorded by large strip electrodes, or by a balloon and transducer, attached to a hind leg of 12 cats.

11 *Pharmacology*

PYROGENIC EFFECT OF S. TYPHOSA LIPOPOLYSACCHARIDE INJECTED INTO THE LATERAL CEREBRAL VENTRICLE IN CATS. U. K. Sheth†*, W. G. Clark*, R. Rosenstein* and H.L. Borison. Dept. of Pharmacology, Univ. of Utah, Salt Lake City.

Body temp. was recorded continuously on a Leeds & Northrup Speedomax in chronic unrestrained cats injected intraventricularly through permanent cannulae, as described by Feldberg and Sherwood. The avg. temp. reading for 8, 9 and 10 a.m. was used as the normal standard for each cat, and the diurnal variation in 8 untreated cats for 25 cat-days was 0.4±0.8°F (avg.±S.D.). Injections were made at 10 a.m. each day. Intraventricular 0.9% NaCl (0.25 ml) did not produce a detectable temp. rise. When pyrogen was given in a constant vol. of 0.25 ml, the threshold pyrexic dose was 0.1 µg (onset-4 hrs, duration-5 hrs); the dose for max. effect was 0.6 µg (onset-1 hr, duration-9 hrs, peak rise-5°F at 4 hrs). No tolerance developed after 8 successive daily intraventricular injections of 0.6 µg. By contrast, daily intravenous injection of 50 µg/kg through an indwelling catheter in the ext. jugular vein, over a 10-day period, resulted in early development of tolerance, as seen by loss of 2nd phase of pyrexia. At the end of this period, intraventricular pyrogen elicited an undiminished response. Intraventricular apomorphin HCl (250 µg) elicited vomiting but not pyrexia. (Supported by Grant No. B-491, Natl. Insts. of Health, PHS) †Fellow of Rockefeller Foundation; Seth, G. S. Medical College, Bombay.

024. BIOLOGICAL REGULATION—Enzyme Action

1 *Physiology*

MICROANALYTICAL TECHNIQUE FOR THE QUANTITATION OF SELECTED ENZYME-SUBSTRATE SYSTEMS. R. L. Davis*, A. H. Lawton, S.R. MacIntyre* and R. F. Chow*. VA Ctr., Bay Pines, Fla. and Johns Hopkins Univ., Baltimore, Md.

Deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) inhibit the microbial adsorption of vit. B_{12} tagged with Cobalt^{60} by the resting cells of Lactobacillus leichmannii ATCC 4797. The magnitude of this inhibition is directly proportional to the amount of RNA or DNA added. With the incorporation of ribonuclease (RNase) or deoxyribonuclease (DNase) into a system containing the respective nucleic acids, Co^{60} tagged vit. B_{12}, resting cells and necessary buffers, a marked increase in microbial adsorption of vit. B_{12} results due to enzymatic reaction. In a series of experiments, increasing amounts of DNase, Rnase as well as of other enzymes whose substrates inhibit microbial adsorption of vit. B_{12} were added to constant amounts of DNA, RNA and other substrates in bioassay system described earlier containing Co^{60} vit. B_{12} and resting cells. The analytical data derived from the microbial uptake response curves demonstrates that with the incorporation of millimicrogram to microgram amounts of substrate, quantitation of the respective enzyme systems can be readily achieved. This technique is applicable to the measurement of selected enzymes in biological systems including blood and urine.

2 *Physiology*

ACETATE ACTIVATING ENZYME ACTIVITY IN LIVERS OF COLD-FASTED RATS. H.M. Korchak and E.J. Masoro (intr. by J.M. Hayman, Jr.) Dept. of Physiology, Tufts Univ. Sch. of Med., Boston, Mass.

Liver slices and homogenates from cold-fasted rats (fasted for 24 hours at 0-2°C) are defective in their ability to oxidize acetate-1-C^{14} to $C^{14}O_2$. In contrast mitochondria from such rats exhibit no significant defect in acetate oxidation. To gain insight into these phenomena the activities of acetate-activating enzyme in livers of normal and cold-fasted animals were investigated. A fall in specific activity was found in the liver homogenates from cold-fasted rats. In the livers of control rats the highest specific activity was found in the particle-free supernatant. This high enzyme activity fell markedly after a "cold-fast". In control mitochondria, the specific activity was lower than in the supernatant and this was essentially unaffected by "cold-fasting". The high level of enzyme activity in the supernatant suggests that acetate activation occurs here and that acetyl CoA is then transported to the mitochondria for oxidation to CO_2. The observed defect in acetate oxidation in liver slices and homogenates from cold-fasted rats is probably linked with a fall in supernatant enzyme. That acetate oxidation by isolated mitochondria is not changed by a "cold-fast" is probably due to the maintenance of a relatively normal mitochondrial level of enzyme.

3 *Physiology*

THE 17β-ESTRADIOL SENSITIVE ENZYMES OF HUMAN CHORIOCARCINOMA. Donald A. Kellogg* (intr. by Roy Hertz.) Natl. Insts. of Health, Bethesda, Md.

Three 17β-estradiol sensitive enzyme systems previously described in human term placenta, a DPN-linked dehydrogenase, a TPN-linked dehydrogenase, and a TPNH-DPN transhydrogenase, have been assayed in homogenates of human choriocarcinoma, a tumor of the placenta, obtained from the patient or maintained in heterologous transplant in the hamster cheek pouch. As with term placenta, the activities were present only in the soluble fraction of the cell. In choriocarcinoma, the specific activities per gram of wet weight or per milligram of protein were lower than in term placenta. Following prolonged chemotherapy these ranged as low as 1% of that in term placenta for the 2 dehydrogenases and 10% for the transhydrogenase. The lack of parallelism in the changes in dehydrogenases and transhydrogenase following therapy suggests that more than one protein system is involved in these 3 reactions. During the accelerated growth phase of heterologously transplanted human choriocarcinoma, hormones or drugs were administered to the host hamster. The effect of such administration on these 3 systems in the choriocarcinoma will be described as well as a general survey of tissues of several species for these reactions.

4 *Physiology*

THE EFFECT OF FEEDING ON SUCCINOXIDASE ACTIVITY IN SELECTED RAT TISSUE. Read R. Nielson*, Anthony J. Steffek*, Alvin F. Rieck and Howard M. Klitgaard. Marquette University School of Medicine, Milwaukee, Wisconsin

The succinoxidase activity was assayed by the manometric and the tetrazolium dye reduction methods in tissue homogenates. All animals were fed for 5 hours each day. Determinations were made at time intervals between 1 and 23 hours after the termination of the feeding period. The non-feeding period in a second group of animals were extended to 52 hours. All tissues studied had their lowest activity 6 hours after feeding and this was used as the reference point of 100%. The highest activity for liver (152%) and kidney (116%) was reached at 23 hours while heart (121%) and skeletal muscle (127%) showed highest activity 1 hour after feeding. Periods after feeding of 27 and 52 hours showed a plateau of activity for liver and skeletal muscle whereas heart and kidney declined steadily after 27 hours. Correlation of the two methods of determining succinoxidase activity was 0.969, 0.413, 0.611 and 0.735 for liver, kidney, heart and skeletal muscle respectively. These results show the importance of controlling fasting and feeding periods in succinoxidase studies. (Supported by U.S.P.H. grant A-1706.)

5 *Physiology*

ACTIVITY OF SELECTED GLYCOLYTIC ENZYMES DURING HIBERNATION. David A. Vaughan and John P. Hannon. Arctic Aeromedical Lab. APO 731, Seattle, Washington.

Assays of the specific activities of phosphorylase, phosphoglucomutase, glucose-6-phosphate dehydrogenase, and glucokinase were made at 30° C on liver and heart tissue of hibernating and non-hibernating Arctic ground squirrels and the laboratory rat. In addition, glucose-6-phosphatase was assayed in liver tissue from both species. When compared to the rat, liver tissue from non-hibernating squirrels exhibited higher levels of phosphorylase and glucose-6-phosphatase. Heart tissue from non-hibernating squirrels was found to have lower phosphorylase and glucose-6-phosphate dehydrogenase than heart tissue from rats. Hibernation had the following effects (1) a decrease in liver phosphorylase and liver glucose-6-phosphate dehydrogenase and (2) an increase in heart glucokinase, with a decrease in heart glucose-6-phosphate dehydrogenase. Liver glucose-6-phosphatase, glucokinase and phosphoglucomutase, as well as heart phosphorylase and phosphoglucomutase were not affected by transition from the awakened state to hibernation. The data indicate that (1) upon hibernating, the squirrels lose, to a great extent, their capacity to metabolize liver glycogen, and (2) in the heart of the hibernating squirrel the ability to metabolize glycogen is maintained while the potential for glucose utilization may be increased.

6 *Biochemistry*

THE pH DEPENDENCE OF THE COMPETITIVE INHIBITION OF FUMARASE. Paul W. Wigler* and Robert A. Alberty. Dept. of Chemistry, University of Wisconsin, Madison, Wis.

In order to obtain further information on the structure and function of the active site of fumarase, the inhibition constants of four competitive inhibitors (succinate and the three isomers of tartrate) have been determined as a function of pH with L-malate as the substrate. The dependence of the inhibition constant (K_I) on pH is given by

$$K_I = K_I'[1 + (H^+)/K_{aE} + K_{bE}/(H^+)]/[1 + (H^+)/K_{aEI} + K_{bEI}/(H^+)]$$

where K_I' is the inhibition constant for the intermediate ionized form of the enzyme, K_{aE} and K_{bE} are the macroscopic acid dissociation constants of the enzymatic site and K_{aEI} and K_{bEI} are similar constants for the inhibitor-enzyme compound. The parameters of the foregoing equation were found to be markedly different for each inhibitor. These differences may be ascribed to the fact that the hydroxyl groups of D and L-tartrate are in the cis configuration while the hydroxyl groups of meso-tartrate are trans. The results are interpreted in terms of a steric model for the active site based on a trans mechanism for the dehydration of L-malate by fumarase.

7 *Biochemistry*

VARIATIONS IN ENZYME STABILITY WITH SUBSTRATES. S. Grisolia, B.K. Joyce* and J. Caravaca.* McIlvain Laboratories, Medical Center, Kansas City, Kansas.

Increased thermal stability of enzymes by substrates has often been observed. We have shown that substrates or cofactors may also decrease thermal stability. This is a relatively common phenomenon since of some 20 enzymes tested about half are not affected or are protected by substrates, while the other half are more susceptible to heat in the presence of certain substrates or cofactors. Interaction of substrate with a catalytic group may protect this or other groups and in addition, by changing the geometry of the enzyme, make the enzyme more susceptible to inactivation by heat, ultraviolet light, X-rays, ultrasonic waves, etc. Examples of these effects on lactic, glyceraldehyde phosphate, malic and alcohol dehydrogenases, and of lyophilization on apparent activity and on reactivation of malic dehydrogenase will be presented. The increased lability by substrates may reflect a general biological principle and may provide a working hypothesis for certain biochemical and pharmacological problems.

8 *Biochemistry*

Characterization of an Unusually Heat Resistant Inorganic Pyrophosphatase from Azotobacter agilis. Emmett J. Johnson (intr. by Virginia R. Williams.) Univ. of Miss. School of Med., Jackson, Miss.

An enzyme has been discovered in Azotobacter agilis which resists heating at 75°C for at least 2 hrs. in the presence, but not in the absence, of magnesium. It has a requirement for magnesium, and a broad pH range on the alkaline side, from pH 7.0 to 10.5. It appears to be specific for inorganic pyrophosphate having no activity on ADP, ATP, DPN, or TPN. Treatment with 1% crude trypsin, under optimum conditions for trypsin activity, denatures the enzyme in the absence, but not in the presence of magnesium. Treatment with crystalline trypsin does not affect this enzyme under any conditions. Magnesium also stabilizes hydrogen bonds of the enzyme against disruption by 6 and 9 M urea. Magnesium provides additional stabilization to the enzyme upon treatment with urea and thioglycollate combined. Thioglycollate and cysteine alone have no affect on the enzyme. The denaturation by urea and thioglycollate combined, in the absence of magnesium, appears to be irreversible. The evidence presented here indicates that this enzyme possesses an unusual type of bonding and herein may reside, at least in part, the molecular basis of heat stability.

XUM

9 **Biochemistry**

PROPERTIES AND MECHANISM OF ACTION OF β-THIOLPYRUVATE TRANSSULFURASE. Dean W. Fanshier* and Ernest Kun. Dept. Pharmacology and Exp. Therapeutics, The Univ. of California Medical Center, San Francisco, Calif.

The protein fraction containing thiolpyruvate transsulfurase of rat liver (Biochim. Biophys. Acta 32, 338, 1959) was subjected to gradient elution chromatography on DEAE cellulose and separated into copper protein fractions, only one of which had enzymic property. The specific activity of the enzyme was increased 10-fold over the one obtained by electrophoretic fractionation. Enzyme inhibition by Cu complexing agents (8-hydroxyquinoline, o-phenanthroline) and substrate analogues as well as physical properties of the enzyme were determined. (Supported by USPH grants H-2897 and C-3211).

10 **Biochemistry**

SERUM ENZYMES AND BIOCHEMICAL INDIVIDUALITY. Walter Frajola, Erica Meyer-Arendt* and James Waltz*. H. A. Hoster Res. Lab. and Dept. of Pathology, Ohio State Univ., Columbus, Ohio.

Roger J. Williams in his book, "Biochemical Individuality", indicated that each individual may be characterized by a distinctive pattern of serum enzyme levels. In order to test this hypothesis several serum enzyme activities and the levels of total and free vitamin B_{12} were determined on each of many serum samples. The enzymes assayed were: phosphohexose isomerase, glutamic-oxalacetic transaminase, glutamic-pyruvic transaminase, lactic dehydrogenase, malic dehydrogenase, alkaline phosphatase, leucyl aminopeptidase, aldolase, and acetylcholinesterase. Sera from seven pairs of identical twins, sera of siblings and parents of the twins, and sera of about fifty other healthy individuals were assayed. Assays were repeated at various intervals of time from two to ten days for periods up to six months. The results indicate that serum enzyme patterns are remarkably specific and are biochemical characteristics of the individual. (Supported in part by S. J. Roessler Memorial Medical Scholarship Fund and Contract AF 33(616)-5537 Task No. 71836 Wright Air Development Center).

11 **Biochemistry**

IN VITRO INHIBITION OF PHOSPHOGLUCOMUTASE BY TOCOPHEROL. Harris Rosenkrantz and Roland C. Laferte*. Worcester Foundation for Exptl. Biol., Shrewsbury, Mass.

Although anaerobic glycolysis in skeletal muscle from tocopherol depleted rabbits appeared normal (J. Biol. Chem. 234: 35, 1959) glycogen levels sometimes were depressed. Exogenous tocopherol caused a greater decrease in glycogen levels in muscle from control and deficient animals. A similar finding had been reported by Zierler et al. (Bull. Johns Hopkins Hosp. 92: 41, 1953) for diaphragm of rat given α-tocopheryl phosphate. They concluded from indirect evidence that glycogen deposition was partially blocked at the phosphoglucomutase level. In the present study D-α-tocopherol in propylene glycol was introduced into purified enzyme systems to establish this point. Hexokinase activity was estimated via glucose disappearance by the method of Nelson; glucose-6-phosphate dehydrogenase by TPNH absorption at 340 mμ; phosphorylase by disappearance of inorganic phosphate and phosphoglucomutase by the decrease in glucose-1-phosphate levels according to Bodansky. One mg. of tocopherol inhibited phosphoglucomutase 50-100%. Glucose-6-phosphate dehydrogenase, phosphorylase and hexokinase were not inhibited. Tocopherol may aid muscle activity by favoring glycolysis to glycogenesis. (Supported by Muscular Dystrophy Associations of America, Inc.).

12 **Biochemistry**

STUDIES ON ATP-TRANSPHOSPHORYLASES. Theodore A. Mahowald* and Stephen A. Kuby, Institute for Enzyme Research, University of Wisconsin, Madison.

Amino acid analyses for the crystalline enzymes, myokinase and ATP-creatine transphosphorylase, have been conducted by two methods (Levy and Stein-Moore). A search for an N-terminal group in both proteins has been negative by either the dinitrofluorobenzene (DNFB) or phenylisothiocyanate procedures employing native and guanidine denatured proteins. ATP-creatine transphosphorylase and also myokinase are stoichiometrically inactivated by 2 equivalents of DNFB, a fact which may be correlated with (a) equilibrium binding data for their substrates (Federation Proceedings, 267, 18 (1959); (b) inhibition by silver for myokinase; and (c) silver amperometric titration of myokinase for -SH groups (J. Biol. Chem., 551, 226 (1957)). Employing the subtractive approach and the chromatographic procedure of Stein and Moore, analyses of both proteins before and after treatment by 2 equivalents of DNFB have revealed a decrease in the value of 1/2 cystine (measured either as cystine or after oxidation to cysteic acid) to the extent of roughly 2 residues.

13 **Biochemistry**

REACTIVITY AND CATALYSIS OF REACTIONS OF THE SERINE HYDROXYL GROUP AND OF O-ACYL SERINES. B. W. Anderson*, E. H. Cordes* and W. P. Jencks, Brandeis Univ., Waltham, Mass.

The hydroxyl group of N-acetylserineamide, a model for part of the active site of certain enzymes, is much less reactive than chymotrypsin, but is 310 to 1340 times as reactive as the hydroxyl group of water or simple alcohols in base-catalyzed reactions with p-nitrophenyl acetate and acetylimidazole. This high nucleophilic reactivity as well as the "low-energy" nature of the product make the serine hydroxyl group a particularly well-fitted reagent for enzymatic attack on "low-energy" substrates.

The reactions of N,O-diacetylserineamide, a model for the acylated active site of chymotrypsin, with water at 100° and with hydroxylamine at 25° are catalyzed by imidazole. The latter reaction is markedly slower in D_2O, showing that imidazole acts as a true general base catalyst, rather than a nucleophilic catalyst, in this reaction of a "low-energy" ester. The rate constants for the base-catalyzed hydrolysis at 25° of N,O-diacetylserineamide and of acetyl chymotrypsin in 8 M urea (Dixon et al. JACS 78, 4810, 1956) are 16.2 and 12.3 $M^{-1}min.^{-1}$; for hydroxylaminolysis they are 1380 and 1230 $M^{-2}min.^{-1}$, respectively. This provides further evidence that the primary site of acylation of chymotrypsin is a serine hydroxyl group.

14 **Biochemistry**

AMINO ACIDS AT THE ACTIVE SITE OF A SINGLE DISPLACEMENT ENZYME. John A. Thoma* and D. E. Koshland, Jr. Biol. Dept., Brookhaven National Laboratory, Upton, N. Y.

The labeling of the active site of double displacement enzymes has started to clarify the roles of the amino acids during the enzyme action, but similar information is not available for single displacement enzymes. Accordingly a study of beta amylase has been initiated to identify the amino acids at its active site. Optical rotation studies on maltotetraose showed that quantitative inversion occurs during the enzyme action. A plot of -log Vm vs. pH for native beta amylase shows a group with pK of 4.3 must be present as a base and a group with pK 7.1 must be present as an acid for optimum activity. When the cysteine groups of the enzyme are quantitatively alkylated with iodoacetamide the enzyme loses 98% of its activity but still shows essentially the same Vm-pH profile. That the sulfhydryl group is at the active site is shown by the fact that cyclohexaamylose, a competitive inhibitor, protects against iodoacetamide inactivation. At least three groups--carboxyl, imidazole, and sulfhydryl--are involved in the catalytic action of beta amylase. (Research carried out at Brookhaven National Laboratory under the auspices of the U. S. Atomic Energy Commission.)

15 *Biochemistry*

CRYSTALLINE Δ^5-3-KETOSTEROID ISOMERASE. F. S. Kawahara* and P. Talalay, University of Chicago.

A steroid isomerase converts Δ^5-3-ketosteroids into Δ^4-3-ketosteroids by promoting an intramolecular transfer of hydrogen from C-4 to C-6. This steroid-induced enzyme has been purified 3000-fold and crystallized from Pseudomonas testosteroni. The twice crystallized enzyme has remarkably high catalytic activity, since 1 mg of this protein promotes the isomerization of 169,000 μmoles of 5-androstene-3,17-dione per min. at 25°. The properties of the enzyme have been studied, especially with respect to solubility, kinetics, specificity, stability and inhibition. The isomerase is resistant to sulfhydryl group combining reagents. Cysteine or cystine was not detected in hydrolysates of the enzyme. Various metal ions or metal chelators were without influence on the activity. No evidence could be obtained for the presence of a prosthetic group; although the spectrum showed the low ratio of 1.2 for the light absorption at 280 to that at 260 mμ. The enzyme undergoes a partially reversible denaturation in 6-10 M urea to a relatively stable product which has 1.5% the activity of the native enzyme and which, unlike the native enzyme, is readily digested by trypsin.

16 *Biochemistry*

THE ALCOHOL-ACETALDEHYDE DEHYDROGENASE SYSTEMS IN THE LIVERS OF ALCOHOL-TREATED RATS. R. M. Dajani* and James M. Orten, Department of Physiological Chemistry, Wayne State University College of Medicine, Detroit.

Previous work in this laboratory has demonstrated that isolated liver cells from alcohol-treated rats utilize ethanol by way of the citric acid cycle more readily than do those of control rats but utilize acetate to a lesser extent. This finding prompted the present study of the alcohol-acetaldehyde dehydrogenase systems in livers of such animals maintained on known amounts of purified diets. Alcohol dehydrogenase was determined by the method of Bonnichsen and Brink (Methods in Enzymology, I: 495, 1955) and acetaldehyde dehydrogenase is being determined by a modification of the method of Racker (J. Biol. Chem., 177: 883, 1949). It was found that the alcohol dehydrogenase activity of the livers of the alcohol-treated rats was considerably higher than that of the controls and there appears to be some difference in the aldehyde activity of the livers of the two groups. The latter observation is being checked by the Warburg technique. The implications of these findings to alcohol metabolism and their relation to food intake will be discussed. (Supported by a grant from the Michigan State Board of Alcoholism).

17 *Biochemistry*

PURIFICATION OF GLUTAMIC-OXALACETATE TRANSAMINASE FROM SKIN. G. Thomas Passananti* and Walter D. Block, Dept. of Dermatology, Medical School, University of Michigan, Ann Arbor.

Although the presence of transaminase in rat skin has been known since 1952, no attempt has been made to purify the enzyme or to study its metabolic role in this tissue. Rat skin powder was extracted with phosphate buffer containing KCl and the supernatant obtained after centrifugation contained the total salt extractable protein fractions. Preliminary purification by dialysis against dilute buffer and subsequent centrifugation yielded two fractions: (1) the salt extractable-water insoluble proteins and (2) the salt extractable-water soluble proteins. The latter fraction, containing transaminase activity, was applied to a column of DEAE-cellulose and the proteins separated by gradient elution. The glutamic-oxalacetic transaminase haloenzyme was eluted in a single sharp peak with a protein corresponding to γ-globulin in its electrophoretic properties. Further purification using $(NH_4)_2SO_4$ fractionations gave a product of high purity (170,000 units/mg). The enzyme, purified 500-fold, did not transaminate a glutamic-pyruvic system and was stimulated by addition of pyridoxal phosphate. Physico-chemical studies on the purified enzyme are in progress.

18 *Biochemistry*

CHLOROPEROXIDASE: A COMPONENT OF THE β-KETOADIPATE CHLORINASE SYSTEM. Paul Shaw and Jonathan Beckwith (intr. by K. Bloch) Chemistry Department, Harvard University, Cambridge, Mass.

Previous communications from this laboratory have described the preparation of a soluble extract from mycelial powders of Caldariomyces fumago which catalyzes the formation of δ-chlorolevulinate from β-ketoadipate and chloride ion. Purification of the C. fumago extracts revealed a multiple enzyme and a heat stable factor requirement for catalytic activity in this reaction. The heat stable factor has been identified as a polysaccharide which couples with a hydrolytic enzyme and glucose oxidase to function in a hydrogen peroxide generating system. Evidence has been obtained that an additional enzymatic component catalyzes the peroxidation of chloride ion in the presence of the enzymatically generated hydrogen peroxide. The chloroperoxidase has been purified 100 fold by calcium phosphate gel fractionation and chromatography on DEAE cellulose. Chloroperoxidase may be assayed independently from the β-ketoadipate chlorinase system by measuring the chloride ion dependent decomposition of hydrogen peroxide. In the presence of catalase, which competes with the chloroperoxidase for hydrogen peroxide, the previously observed stoichiometry of ½ mole of oxygen uptake per mole of δ-chlorolevulinate formed is explained when corrections are made for the oxidation of glucose and gluconic acid.

19 *Biochemistry*

THE USE OF KINETIC ISOTOPE EFFECTS IN STUDIES OF METABOLIC REGULATION. Irwin A. Rose, Dept. of Biochemistry, Yale Medical School, New Haven, Conn.

The action of yeast glucose 6-P dehydrogenase on normal and 1-deutero glucose 6-P was studied. The V_{max} was half as great with the deuterated substrate. Over the pH range 6-9.5 the K_m at saturating TPN concentrations was 1.6 to 1.7 times greater for the normal substrate. The K_m of TPN was the same with the two forms of glucose 6-P. The isotope effect on the rate occurred at concentrations of TPN much below half-saturation. Isotope effects of the same magnitude were found also with the enzyme isolated from E. coli, human erythrocytes, and L. mesenteroides. Since the isotope effect occurs over a broad range of pH, and glucose 6-P and TPN concentrations it may be used to determine whether the dehydrogenase step is rate-limiting in the oxidation of glucose by the hexose-monophosphate pathway. With human red blood cells, 1D,1C^{14}-glucose was found to give rise to labeled CO_2 at one-half the rate for 1H,1C^{14}-glucose. A much smaller isotope effect was encountered if methylene blue was provided to stimulate O_2 uptake. These and effects with other cells will be used to illustrate the application of kinetic isotope effects to the study of rate-control mechanisms in the intact cell.

20 *Biochemistry*

STUDIES ON THE MECHANISM OF FORMATE ACTIVATION BY TETRAHYDROFOLIC ACID FORMYLASE. Richard H. Himes* and Jesse C. Rabinowitz. Dept. of Biochemistry, Univ. of Calif., Berkeley.

THF-formylase catalyzes the reaction: THF + formate + ATP ⇌ 10-formyl-THF + ADP + iP. The mechanism of the reaction was investigated by using crystalline enzyme from Clostridium cylindrosporum. The formation of ADP from ATP was determined in the presence of the phosphoenolpyruvate kinase-lactic dehydrogenase system. The rate of ADP formation in the presence of ATP and either formate or THF is negligible compared to the rate observed in the presence of both substrates. All the reactants are required for the incorporation of iP^{32} into ATP. The incorporation of the P^{32} of adenosine-P-P^{32} into ATP, however, occurred in the absence of both formate and THF. This exchange is not caused by adenylate kinase since the product formed was shown to be adenosine-P-P^{32}-P. The enzyme is also free of nucleoside diphosphokinase activity. Neither formate nor THF alone affected the rate of the ADP-ATP exchange. These results fail to support reaction mechanisms in which either formate or THF reacts initially with ATP to form a phosphorylated derivative.

XUM

21 *Biochemistry*

MECHANISM OF ENZYMATIC ISOMERIZATION OF A PRIMARY TO SECONDARY ALCOHOL COUPLED TO PHOSPHATE ELIMINATION. Martin Flavin and Clarence Slaughter.* Enzyme Section, National Heart Institute, Bethesda, Md.

A pyridoxal-P dependent enzyme catalyzing the stoichiometric formation of threonine and Pi from P-homoserine has been purified 500-fold from Neurospora. Reversibility cannot be demonstrated with threonine-C^{14}. Incubation in H_2O^{18} leads to the incorporation of one solvent O into threonine and none into Pi. Since homoserine and P-threonine are not substrates, Pi must be removed by elimination rather than hydrolysis (B.B.A.36:554). In 100 % D_2O, exactly 2 solvent D atoms are incorporated into threonine. Degradations show D in the α position to be 0.98 atoms; β + γ, 0.99; γ, 0.92. The low incorporation of solvent tritium (Fed.Proc.18: 226) apparently reflects addition to an intermediate of protons 40 times faster than tritium ions. Elimination of H_3PO_4 shifting of double bonds in the resultant enzyme-bound Schiff base of vinylglycine, and addition of water, are all potentiated by coenzyme in the proposed mechanism. Homoserine kinase has been tentatively identified in Hughes Press extracts of Neurospora.

22 *Biochemistry*

MAMMALIAN THIOGLYCOSIDASE AND SULFHYDRYL GROUP FUNCTION. Irving Goodman, Ludwig Salce* and Robert B. Hiatt*, Depts. of Biochemistry and Surgery, College of Physicians & Surgeons, Columbia Univ., New York.

Previous studies (Science, 130:450, 1959) demonstrated the presence, in mammalian tissues of an enzyme system which catalyzed the hydrolysis of a number of synthetic thioglycosides including 6-purinyl-β-D-glucothiopyranoside and the corresponding -9-ribosyl and 2-amino-9-ribosylpurine nucleosides. As part of a study to determine the role of this enzyme in natural biochemical systems, enzyme fractions were prepared from various sources including rat intestine and excised segments of human ileum and colon. Enzyme activities were determined using as substrates a series of new synthetic thioglucosides of naturally occurring SH- containing compounds including -β-(L-cysteinyl)-β-D-glucothiopyranoside, γ-(L-Homocysteinyl)-β-D-glucothiopyranoside, and Glutathione-β-D-glucothiopyranoside. These derivatives are shown to be distinct from the known thiazolines and thiazolidines, and serve as excellent substrates for the above mammalian thioglycosidase preparations. Possible roles for the sulfhydryl group in the linkage of carbohydrates to proteins and in the biosynthesis of carbohydrates are suggested.

23 *Biochemistry*

ALKALINE PHOSPHATASE FORMATION IN B. SUBTILIS. C. Anagnostopoulos (intr. by J. Spizizen.) Dept. of Microbiology, Western Reserve Univ., Cleveland.

Alkaline phosphatase in a number of B. subtilis strains was found to be a repressible enzyme as has been reported for this enzyme in E. coli (Torriani, Feder. Proc. 18, 1344, 1959). Production of the enzyme in B. subtilis starts when the concentration of inorganic phosphate (Pi) in the medium is lower than 10^{-5}M. The rate of formation reaches its maximum value about three hours after the exhaustion of all exogenous phosphate. Optimal conditions for formation include $Mg^{++}(10^{-2}M)$ and a mixture of amino acids. In auxotrophic strains the response to increasing concentrations of the required amino acid is similar for phosphatase formation and growth. The alkaline phosphatase of B. subtilis behaves as a non-specific phosphomonoesterase with a pH opt. of 10.2 (for p-nitrophenyl-phosphate in ethanolamine buffer). It is activated by Mg^{++} and inhibited by low concentrations of Pi and arsenate. The enzyme also possesses phosphotransferase activity. By UV irradiation mutants have been obtained which cannot form alkaline phosphatase. These mutants can be transformed with high efficiency by wild type DNA to organisms able to synthesize this enzyme. Studies on the purification of the enzyme will be reported.

24 *Biochemistry*

THE ENZYMATIC OXIDATION OF ACETOL BY LIVER. Kenneth H. Shull* and O. Neal Miller. Tulane Univ. Sch. of Med., New Orleans, La.

Particulate-free supernatant fractions were prepared from 10% rat liver homogenates. The supernatant fractions were dialyzed against 0.01 M phosphate buffer pH 7.4 for 24 hrs. before being employed as the enzyme source. The oxidation of acetol (monohydroxyacetone) was assayed by the increase in optical density at 340 mμ due to the reduction of DPN. The oxidation of acetol was studied at pH values of 8.5, 9.0, 9.5, 10.0 and 10.5 using 0.2 M glycine-NaOH buffers and at pH 11 using 0.2 M pyrophosphate buffer. Values for Km and Vmax were calculated from the conventional Lineweaver-Burk plots at the above mentioned hydrogen ion concentrations. The greatest enzymatic activity was obtained at pH 10.5. Acetol phosphate was not oxidized by this system at any of the hydrogen ion concentrations studied. p-chloro-mercuribenzoate, at a concentration and under conditions which completely inhibits alcohol and lactic dehydrogenases, had no effect on the enzyme under study. Although the product of the oxidation of acetol has as yet not been determined, it appears that methylglyoxal (pyruvaldehyde) is the most likely one. Removal of two hydrogen atoms from the primary alcohol group of acetol would produce this compound.

25 *Biochemistry*

LACTATE OXIDATION BY HEART MUSCLE MITOCHONDRIA. P. D. Deshpande*, D. Hickman*, and R. W. Von Korff, Medical School, University of Minnesota, Minneapolis, Minnesota.

Oxidation of L(+) lactate (I) to CO_2 by washed heart muscle mitochondria is observed when the medium is supplemented with LDH and DPN. The well documented oxidative utilization of I by intact heart is believed to occur after prior conversion to pyruvate requiring continued reoxidation of the DPNH. In contrast to the slow mitochondrial oxidation of DPNH frequently reported, Ziegler et al recently have stated that "undamaged" mitochondria do not oxidize externally formed DPNH. If the latter is correct the mechanism of I oxidation in heart becomes uncertain and oxidation observed with mitochondria may be an artifact. These questions are of concern in using reconstructed systems for studies of intracellular control mechanisms. It will be shown that reasonably uniform mitochondria can be obtained using published isolation procedures although, with the electron microscope, morphological alterations are evident. The DPNH oxidase activity is low initially. I-C^{14} is oxidized using the Warburg technique. Correlations of metabolic activity with morphology will be reported. Known alternative routes for oxidation of extramitochondrial DPNH appear to be inadequate to explain I oxidation in heart suggesting that either the DPNH is oxidized by mitochondria in-vivo or several routes function additively or an unknown route may exist.

26 *Biochemistry*

TYROSINE-INDUCED CHANGES OF LIVER ENZYME LEVELS CAUSING p-HYDROXYPHENYLPYRUVIC ACID EXCRETION. M. N. D. Goswami* and W. E. Knox. Cancer Research Institute, New England Deaconess Hospital and Harvard Medical School, Boston, Mass.

L-Tyrosine fed to scorbutic as well as to normal guinea pigs not given excess ascorbic acid caused excretion of p-hydroxyphenylpyruvate (pHPP). The accumulation and excretion of pHPP was referable to tyrosine-induced changes in liver enzyme activities occurring within a few hours: a 2-fold increase of the tyrosine-α-ketoglutarate transaminase which forms pHPP, and a 80 percent decrease of pHPP oxidase which degrades pHPP. The normal transaminase to oxidase activity ratio of 0.3 became 3 or 4 after tyrosine feeding. Ascorbic acid in vivo prevented the decrease in pHPP oxidase and also the excretion of pHPP. The main enzymic reason for pHPP excretion after tyrosine feeding is therefore inhibition or decreased amount of pHPP oxidase. Ascorbic acid given after tyrosine restored the depressed oxidase activity to that of control animals after a time lag. The slow in vivo effect of ascorbic acid and our inability so far to reactivate in vitro the depressed pHPP oxidase by ascorbic acid and other means suggested the possibility of a decreased amount of pHPP oxidase in the livers as a result of tyrosine feeding (Supported by PHS and AEC).

27 *Biochemistry*

THE SOURCE OF HYDROGEN IN FATTY ACID SYNTHESIS. J. M. Lowenstein, J. Roberts* and S. Cordes*. Grad. Dept. of Biochemistry, Brandeis Univ., Waltham, Mass.

Comparisons of the rates of $C^{14}O_2$ output from tissue slices incubated with glucose labelled in the C-1 or C-6 positions have been interpreted as evidence that the pentose phosphate pathway is the major source of the hydrogen which goes to reduce TPN. By implication the pentose phosphate pathway is believed to be the main source of hydrogen in reductive biosyntheses. We wish to report measurements of the incorporation of tritium (T) into the fatty acids of fats synthesized by rat tissue slices suspended in Krebs-Ringer-bicarbonate solution. Acetate and D-glucose-1-T (G-1-T) or D,L-lactate-2-T (L-2-T) were incubated with liver slices from normal, starved, and starved-and-refed rats. Fatty acid synthesis was measured in parallel by the incorporation of C^{14}-acetate into saponifiable fats. A greater incorporation of tritium into fatty acids was observed with L-2-T than with G-1-T. The amounts of hydrogen contributed to fatty acid synthesis by L-2-T and G-1-T rise in starvation and fall on refeeding. The results will be discussed in terms of the pyridine nucleotide specificities of lactic dehydrogenase and the fatty acid synthesizing enzymes, as well as the isotope effects of tritium in dehydrogenase reactions.

28 *Biochemistry*

KINETICS OF CATALASE SYNTHESIS AND DESTRUCTION IN VIVO. V. E. Price and M. Rechcigl, Jr.*, Laboratory of Biochemistry, National Cancer Institute, Bethesda, Maryland.

Previous studies with Fe^{59} (Absts. of Paper, 134th Meeting, Amer. Chem. Soc., 11C, 1958) have shown that 3-amino-1,2,4-triazole acts by destroying catalase and that the subsequent return of catalase activity results from the synthesis of new enzyme at a rate similar to that in control animals. Kinetic studies have been carried out on the curve of catalase return, and the equation derived will be presented. Comparative data on the rate of catalase synthesis and destruction in liver and kidney of normal rats after injection with aminotriazole, 1g./kg., show that although the rate of synthesis is nearly 4 times higher in the liver than in the kidney (24.5 and 6.6 μg. per hour per g. of tissue, respectively); the fraction of catalase molecules being destroyed per unit time is nearly the same (2.3% and 2.1% per hour, respectively) in both tissues.

Similar studies were carried out on starving animals and although the total amount of catalase in the liver and kidney fell progressively as the total weight and nitrogen of the liver decreased, the rate of catalase synthesis per gram of tissue remained constant. It is suggested that in starvation the animal may control the rate of catalase synthesis by varying the number of synthesizing units, but that the units present at any given time may function at a normal rate.

29 *Biochemistry*

INHIBITION OF ENZYME ACTIVITY BY UREA. K. V. Rajagopalan,* Irwin Fridovich and Philip Handler. Duke Univ., Durham, N. C.

At concentrations insufficient to denature proteins, urea has been found to inhibit a wide variety of enzymes. For those instances studied to date, inhibition has invariably been competitive with respect to organic substrates and non-competitive or uncompetitive with respect to inorganic substrates. Thus, urea competitively inhibits the following (K_I in parentheses): xanthine oxidase (0.2M against both purines and aldehydes), liver aldehyde oxidase (0.8M against N-methylnicotinamide and salicylaldehyde), lactic dehydrogenase (0.6M), acid phosphatase (1.5M), histidase (0.7M), uricase (2.2M), tyrosinase (1.7M), L-amino acid oxidase (1.4M). Either non-competitive or uncompetitive inhibition was found with catalase, rhodanese, inorganic pyrophosphatase and sulfite-cytochrome c reductase. Chymotrypsin, hexokinase and ribonuclease were unaffected at concentrations below 3M. Guanidine is 10-80 times as effective as urea as a competitive inhibitor in those instances studied. Klotz has attributed the inhibition by urea of dye-binding to serum albumin to interference with water-lattice formation on the protein surface. However, glycine and LiBr both proved to be non-competitive inhibitors of xanthine oxidase. Whereas K_m of xanthine oxidase for xanthine varies from 0.4×10^{-6}M at 7° to 6.7×10^{-6}M at 40°, K_I for urea is essentially constant over this temperature range.

30 *Biochemistry*

CONTROL OF D-ARABITOL DEHYDROGENASE AND ETHANOL DEHYDROGENASE LEVELS IN AEROBACTER AEROGENES. Edmund C. C. Lin, Peter McPhedran, and Barbara B. Sommer (intr. by H. C. Trimble) Harvard Medical School, Boston, Massachusetts.

A DPN-linked dehydrogenase has been found which converts D-arabitol to D-xylulose and D-mannitol to D-fructose. It is induced by D-arabitol but not by D-mannitol, although the latter supports growth and is an active substrate in vitro. Moreover, D-mannitol represses the induction of the enzyme by D-arabitol. High levels of this enzyme are induced only under anaerobic conditions similarly to glycerol dehydrogenase (Lin, Levin and Magasanik, J. Biol. Chem., in press). This suggests that D-arabitol dehydrogenase is important only for the fermentation of D-arabitol. The terminal enzyme involved in the anaerobic metabolism of polyhydric alcohols is probably another DPN-linked enzyme, ethanol dehydrogenase. Anaerobic growth increases the level of this enzyme several fold. The activities of ethanol dehydrogenase of cells grown anaerobically in D-mannitol, D-arabitol and glycerol are respectively 0.11, 0.23, and 0.37 units/mg cellular protein. Addition of fumarate as a hydrogen acceptor in the glycerol medium reduces the ethanol dehydrogenase level to 0.10. Therefore, the level of the enzyme is apparently controlled by the oxidation levels of the carbon sources.

31 *Biochemistry*

FORMATION OF TPN-REQUIRING GLYCERALDEHYDE PHOSPHATE DEHYDROGENASE DURING THE DEVELOPMENT OF CHLOROPLASTS IN EUGLENA GRACILIS. George Brawerman and Nancy Konigsberg*. College of Physicians and Surgeons, Columbia Univ., New York.

It has been shown that the green Euglena cells possess a TPN-requiring glyceraldehyde phosphate dehydrogenase (TPN-GPD), in addition to the DPN-requiring enzyme. The dark-grown, colorless cells contain only the DPN enzyme. We have studied the formation of the TPN enzyme in relation to the production of chloroplasts in Euglena. In resting colorless cells exposed to light, the course of production of the TPN-GPD follows fairly closely that of chlorophyll. Removal of light during this process results in a cessation of both enzyme production and chlorophyll synthesis. Cultivation of the green cells at 34° in the light produces an inhibition of chloroplast synthesis, and return of the cells to a lower temperature brings about a rapid resumption of chloroplast synthesis after a lag period of one to two days. The production of the TPN-GPD follows very closely that of chloroplasts, both during the inhibition and the recovery. The synthesis of the DPN enzyme, on the other hand, is linked to the multiplication of the cells. The results indicate a close connection between TPN-GPD synthesis and chloroplast production. (Supported by the American Cancer Society.)

32 *Biochemistry*

AN ESTERASE FROM THE HOG PANCREAS. E. GJESSING and J. C. HARTNETT*. College of Med., Univ. of Vermont, Burlington, Vt.

Purification and study of an esterase present as a zymogen or proesterase in hog pancreas (J. Biol. Chem. 234 1098 1959) have been made. Substrates for the esterase are esters of hydroxy-benzoic acid. Aqueous extracts of acetone ether dried preparations of fresh pancreas have been used in the purification of the proesterase. Such extracts were fractionated on a cellulose exchanger and yielded a component which became active upon incubation with trypsin. Purification of the active esterase has been attained using pH 4.0 extracts and an anionic cellulose exchanger. The active esterase has a high affinity for the exchanger, a property that has been utilized in the purification. Preparations of the active esterase have proteolitic activity (casein) and catalyze the hydrolysis of N-acetyl-tyrosine ethylester. The specific activity for esters of hydroxybenzoic acid of the preparations, are fifty to a hundredfold that of crystalline alpha-Chymotrypsin and Chymotrypsin-B. The investigations to date indicate that the esterase may be a proteolytic enzyme as well.

XUM

33 *Biochemistry*

HORMONAL AND DIETARY EFFECTS ON HEPATIC CARBOHYDRATE ENZYMES. George Weber*, James Ashmore and Gouri Banerjee* Depts. of Biochem. and Microbiol., Indiana Univ. Med. School, Indianapolis, Ind.

The effect of hormonal and dietary factors was examined on rat liver enzymes involved in glucose-6-phosphate utilization. Enzymes were assayed in supernatant fluid obtained by centrifuging the homogenates at 100,000 g for 30 mins. at 0°C. Enzymatic activities were expressed per average cell and per 100 gram body weight. In 6-day fasted normal rats hepatic glucose-6-phosphatase and glucose-6-phosphate dehydrogenase decreased to 60 and to 10% respectively. After 1-day refeeding glucose-6-phosphatase returned to normal and dehydrogenase was increased to 200%. In hypophysectomized rats enzymes were reduced in 1-day fasting to levels reached in normal rats only after a 6-day starvation; however, 1-day refeeding failed to result in any restoration of these carbohydrate enzymes. In dietary experiments high fructose diet and control synthetic diet resulted in increased shunt enzyme and lactic dehydrogenase activities above the enzyme levels of lab-ration fed normal rats. There was no increase when adrenalectomized animals were placed on synthetic or high fructose diet. (Supported by U.S. Public Health Grant No. A2701C).

34 *Pharmacology*

Effect of serotonin on glycolysis in homogenates from the liver fluke Fasciola hepatica. Tag E. Mansour and June S. Menard*. Dept. of Pharmacology, Louisiana State University Medical School, New Orleans, La.

Stimulation of rhythmical movement of the liver fluke by serotonin resulted in increased rates of glucose uptake and of lactic acid production (Mansour, J.P.E.T. 126, 212, 1959). Glucose utilization and lactic acid production by homogenates of flukes were much higher when the intact parasites had been preincubated with serotonin. Stimulation of lactic acid production by serotonin was also observed when glucose-6-phosphate or fructose-6-phosphate was used as substrate but less with fructose diphosphate suggesting that phosphofructokinase activity was increased. While control homogenates had low phosphofructokinase activity it was markedly increased in homogenates from serotonin treated flukes. Furthermore, heated extracts (100°C;5 min.) from serotonin treated flukes as well as serotonin or adenosine-3',5'-phosphate (synthesized chemically or by fluke particles) increased phosphofructokinase activity in homogenates from control flukes. Since under our assay conditions phosphofructokinase limits the rate of glycolysis of fluke homogenates, the stimulation of glycolysis of intact worms by serotonin is possibly due, at least in part, to the increased activity of this enzyme. (Supported by N.I.H. grants E-1386 and E-668).

35 *Pharmacology*

THE IMPORTANCE OF TPN-DEPENDENT MALATE DEHYDROGENASE IN PROVIDING KREBS CYCLE INTERMEDIATES. Walter M. Fitch (intr. by Avram Goldstein) Stanford U., Stanford, Calif.

Fitch and Chaikoff (J. Biol. Chem., in press) studied the effect of substituting a diet containing 60% glucose for a stock diet. This produced an 8-fold increase in the activity of the TPN-dependent malate dehydrogenase, which interconverts pyruvate and malate, and a similar increase in a hexosemonophosphate (HMP) shunt dehydrogenase. This suggested that the TPNH produced by the shunt reaction might serve to drive the carboxylation of pyruvate. Thus, by indirectly supplying replacement of Krebs intermediates drawn off by protein synthesizing and nitrogen sparing mechanisms, a basis for HMP shunt activity is provided additional or alternative to its collaborative role in lipogenesis. To demonstrate that metabolic changes occur which are consonant with those changes observed in the enzyme activities, formulae making use of isotope distribution patterns are derived. They clearly demonstrate the direction and relative amounts of material traveling the two paths connecting the glycolytic stream and the Krebs cycle as well as the relative contribution of fat and pyruvate to acetyl-CoA and the equilibration of asymmetric dicarboxylic acids by a back reaction to fumarate. (Supported by USPHS (Nat'l Cancer Inst.) fellowship CF9087)

36 *Pharmacology*

THE ENZYMATIC BIOSYNTHESIS OF MELATONIN. Herbert Weissbach* and Julius Axelrod. Natl. Heart Inst. and Natl. Inst. of Mental Health, Bethesda, Md.

A new hormone, melatonin (n-acetyl-5-methoxytryptamine), isolated from the pineal gland, has been shown to lighten the color of frog melanocytes and block and reverse the darkening actions of the melanocyte stimulating hormones (Lerner et al., J. Am. Chem. Soc. 81: 6084, 1959). We wish to report the presence of an enzyme, hydroxyindole-O-methyltransferase, in cow and monkey pineal glands that forms melatonin by transferring a methyl group from S-adenosylmethionine to n-acetylserotonin (n-acetyl-5-hydroxytryptamine). The product of the enzymatic reaction has been identified as melatonin by its fluorescent, chromatographic, colorimetric and extraction characteristics. Although serotonin was also a substrate for this reaction, it was only 1/10 as active as n-acetylserotonin suggesting the following pathway: serotonin ⟶ n-acetylserotonin ⟶ melatonin. The enzyme from beef pineal glands has been purified 20 fold and appears to be different from other methyl transferring enzymes. The enzyme could not be detected in a variety of other mammalian tissues, and appears to be highly localized in the pineal region.

37 *Pharmacology*

SPECIFIC AND NON-SPECIFIC PHOSPHATASES AT THE INFLAMMATORY SITE. Leonard B. Achor, Raymond F. Geoffroy* and William L. Bowers*. Merck Inst. for Therap. Res., Rahway, N. J.

Granulation tissue obtained from a modified SAS granuloma pouch (Achor, Fed. Proc. 18, 362, 1959) has been shown to contain the following enzymatic types: alkaline and acid phosphatases, ATP-ase, 5-nucleotidase, glucose-6-phosphatase and phosphodiesterase. The alkaline phosphatase (pH optimum: 10.0) content of the tissue is exceeded only by that of kidney. Administration of hydrocortisone (F^{OH}) decreases this activity to 1/4 that of controls. Acid phosphatase (pH optimum: 5.3) levels are not significantly affected by F^{OH}. The hydrolysis of ATP (at pH 7.5) is decreased 30% by hydrocortisone. A comparative distribution study of 5-nucleotidase (pH optimum: 8.8) in the rat revealed more of this enzyme in the granulation tissue than any other excepting liver (equal activity). Other mono-nucleotides (cytidylic, uridylic and inosinic acids) are hydrolyzed at rates comparable to 5-AA. Glucose-6-phosphatase heretofore has been demonstrated only in liver, kidney, small intestine and pancreas. Measurable activity, however, is present in SAS granulation tissue. Liver contains 65% more phosphodiesterase activity than tissue from the SAS granuloma pouch. SAS p-ases differ from those of other tissues in a quantitative sense only. Activity levels are steroid sensitive.

38 *Pharmacology*

NATURE OF THE INHIBITION OF CARBONIC ANHYDRASE BY ACETAZOLAMIDE. Kenneth C. Leibman and Donald Alford (intr. by T. H. Maren.) Dept. of Pharmacology and Therapeutics, Univ. of Florida College of Medicine, Gainesville, Florida

Although carbonic anhydrase-inhibiting drugs have been in use for many years, the nature of the inhibition caused by such drugs has remained undetermined. In this work, the kinetics of carbonic anhydrase inhibition by acetazolamide were studied. Known concentrations of CO_2 were achieved by saturating solutions in the cell of a pH-stat with various CO_2-N_2 mixtures at 5°C. The pH was automatically maintained at 8.0 by titration with NaOH. From the titration curves, rates of uncatalyzed and catalyzed reactions and rates in the presence of acetazolamide were obtained. The enzyme was a purified preparation from erythrocytes. The data indicate that acetazolamide is a non-competitive inhibitor of carbonic anhydrase. K_I is approximately 10^{-8} M. It is of interest that the inhibition of carbonic anhydrase by sulfanilamide has recently been shown to be non-competitive by a somewhat different method (Davis, J. Am. Chem. Soc. 81: 5674 (1959).

025. BIOLOGICAL REGULATION— Feed-back Mechanisms

34 *Pathology*

EPIDERMAL RIBONUCLEASE. J. Tabachnick and R. Freed (intr. by C. Weiss.) A. Einstein Med. Ctr., N. Div., Philadelphia, Pa.

Epidermis from excised albino guinea pig skin was scraped and peeled with a scalpel. 0.1 ml of homogenate (5 to 10%) in 0.25 M sucrose was incubated 15 mins at 37C in universal buffer (final volume 2 ml, 0.1μ) with 2.5 mg/ml dialyzed yeast RNA as substrate. An optimal straight line relationship for enzyme content was obtained for increases up to about 0.18 O.D. at 260 mμ. The pH optimum ranged from 7.0 to 7.7. The curve had no peak in the acid range; about 50% of maximal activity wasobtained at pH 5.2. Autolysis of epidermal RNA showed slight RNase activity (ca. 15%) at about pH 5.2 with an optimum between 7.0 and 8.3. The enzyme is relatively heat-stable after heating 5 mins at 95C, pH 4.5. Addition of 5×10^{-4} M p-hydroxymercuribenzoate produced about 30 to 40% increased activity, indicating that an -SH type RNase inhibitor may be bound to some of the enzyme. Isolation of inhibitor by the procedure of Roth [JBC 231, 1085 (1958)] failed. About 75% of RNase activity was removed from peeled epidermis by 3 washings with 0.25 M sucrose (pH 7.4) prior to homogenization. Hence the enzyme is present mainly in the acellular stratum corneum and/or in the interstitial fluid. The relatively high ratio of alkaline to "acid" RNase is similar to observations on other proliferating tissue. (Supported by A.E.C. grant.)

40 *Pathology*

DISTRIBUTION IN THE RABBIT OF INTRAVENOUSLY ADMINISTERED PAPAIN. J.L. Potter*, G. Weissmann*, R.T. McCluskey and L. Thomas. New York Univ. College of Med., New York.

In small rabbits, administration of papain protease causes widespread depletion of cartilage matrix, provided that the enzyme is injected intravenously in an inactive form. Fully reduced, active papain has no such effect on cartilage in vivo. Because this suggested that prior inactivation favored the entry of circulating papain into cartilage, a specific antiserum was used to trace its distribution following I.V. injection. It was found that active and inactive preparations of papain disappeared from the circulation at approximately equal rates, and that the enzyme could not be consistently demonstrated in cartilage at any stage after an injection of either preparation. However, in vitro studies have shown that depletion of isolated cartilage may occur with concentrations of papain as low as 1 microgram/gm. Also, it was found that while depletion progressed following temporary exposure of isolated cartilage to active or inactive papain in saline, and to inactive papain in serum, cartilage temporarily exposed to active papain in serum remained intact. The findings indicate that in vivo depletion of cartilage by papain is attributable to a small fraction which remains free to pass out of the circulation when the enzyme is injected in an inactive form.

41 *Nutrition*

STIMULATION OF CHOLINE SYNTHESIS BY CYTIDINE TRIPHOSPHATE. C.J. Ackerman and May Chou*. Virginia Polytechnic Inst., Blacksburg, Va.

Cytidine diphosphate ethanolamine (CDP-ethanolamine) is readily synthesized in many tissues and is an important intermediate in the synthesis of phosphiolipids. The possibility that such cytidine derivaties may also function as methyl acceptors leading to the synthesis of choline is being investigated. A modified reineckate procedure was devised for the estimation of choline in 100 mg of tissue. In one typical experiment the incubation media consisted of Tris buffer, pH 7.4; $MgCl_2$; glutathione; phosphodimethylaminoethanol; C^{14}-methylmethionine; and 0.5 ml of a 20% rat liver homogenate in a total volume of 2.2 ml. After 1.5 hrs incubation at 37°, the specific activity of the choline reineckate isolated from this system was 0.08 ± 0.03 cts/μg (average of duplicate samples). The addition of ATP increased the specific activity to 4.61 ± 0.2 cts/μg. When cytidine triphosphate (CTP) and ATP were included in the media, the specific activity was 11.58 ± 0.3 cts/μg. Orthosphosphate enhanced this effect of CTP slightly. When dimethylaminoethanol was used as the methyl acceptor, glutathione, orthophosphate, and CTP were necessary for maximum incorporation of radioactivity into choline.

1 *Physiology*

PARATHYROID ACTIVITY AFTER NEPHRECTOMY AS INDICATED BY CHANGES IN OSTEOCLAST COUNT IN FEMUR. R.J. Toft* and Roy V. Talmage. Rice Institute, Houston, Texas.

Male rats maintained on calcium- and phosphate-free diet were sacrificed at varying times after nephrectomy, following which the femurs were prepared for histological study. A significant increase in numbers of osteoclasts per unit area of femur (subepiphyseal portion) could be detected within 6 hrs. following nephrectomy. The peak (100% increase above normal) was reached 24 hrs. following removal of kidneys. A slight drop occurred during the subsequent 48 hrs., though the count was maintained well above normal. Parathyroidectomy, performed at the time of nephrectomy prevented these osteoclast changes, the count remaining normal. Serum calcium values were not affected by nephrectomy during the first 48 hrs. unless the animals had been maintained for some time on a rigorous calcium-free diet in which case serum values often fell below 10 mg/100 ml. Serum phosphate values rose sharply following nephrectomy and were usually above 16 mgP/100 ml by 48 hrs. Calcium administered by stomach tube at regular intervals following nephrectomy prevented the changes in osteoclast count and had little or no effect on serum calcium while preventing the rise in serum phosphate which otherwise occurred. These results are considered as additional evidence for McLean's "feed-back" principle of parathyroid action.

2 *Physiology*

AN ANALYTICAL STUDY ON THE VASOMOTOR WAVES DUE TO THE INCREASED INTRA-CRANIAL PRESSURE. Kiichi Sagawa (intro. by Allan Grafflin.) Depts. of Physiology, School of Medicine, Univ. of Yokohama, Japan and Univ. Med. Center, Jackson, Miss.

Two theories have been offered to explain the mechanism of mean systemic blood pressure waves which often appear when the intra-cranial pressure (ICP) is elevated to approximately equal the systemic blood pressure level in dogs and rabbits. One is a feedback theory which attributes this phenomenon to feedback oscillation based on the Cushing's cerebral ischemic reflex. The other theory is that the phenomenon is nothing but manifestation of an inherent tendency of the circulatory center to oscillate. In order to investigate the validity of these theories, the properties of Cushing's reflex have been studied analytically in terms of automatic control theory. Using rabbits, the ICP was changed sinusoidally around a fairly low level (80 mm. Hg) with fixed amplitude of 20 mm. Hg but with varied frequency (1 to 9 c.p.m.) and the resulting systemic blood pressure waves were compared with the ICP waves in reference to amplitude ratio and phase lag. The results showed the response to be fairly stable so long as the ICP remained below the mean systemic pressure, but as the ICP was raised higher the system became more and more sensitive, thus suggesting the possibility of feedback oscillation.

3 *Biochemistry*

ADENINE DEFICIENCY IN A HISTIDINE AUXOTROPH. Alexandra Shedlovsky* and Boris Magasanik. Dept. of Bacteriology, Harvard Medical School, Boston, Massachusetts

In the purine cycle responsible for histidine synthesis, ATP and PRPP are condensed to Compd. III which is then split into 4-amino 5-imidazole carboxamide ribonucleotide (AICAR) and imidazole glycerol-P. Histidine exerts feedback inhibition on the enzyme forming Compd. III. Certain auxotrophs require histidine or adenine. This alternative requirement could be due to a partial block in the splitting of Compd. III, because the resulting slow histidine formation would release the feedback, and allow unhindered production of Compd. III; this, coupled with the slow regeneration of AICAR, would lead to adenine depletion. Added adenine would permit growth by relieving this deficiency. Added histidine would permit growth by restoring feedback and thus preventing the adenine drain. This hypothesis is supported by the fact that such a mutant grows slowly in minimal medium and possesses little Compd. III-splitting activity. It excretes a derivative of Compd. III and this excretion is prevented by histidine or by its analogue, thiazole alanine (TA) which mimics the feedback action of histidine (Moyed and Friedman. Science 129, 986; 1959). Furthermore, although it is not incorporated into protein, TA supports rapid growth of this mutant.

XUM

026. BIOLOGICAL REGULATION— Hormone Action

4 *Biochemistry*

HORMONE-MODULATED FEEDBACK CONTROL OF CREATINE BIOSYNTHESIS. James B. Walker. Dept. of Biochemistry, Baylor University College of Medicine, Houston, Texas.

Precise metabolic control of creatine biosynthesis may be inferred from the known constancy of creatinine excretion. We have found that both feedback and hormonal controls are operative in this case. Employing either rats or chicks, we have observed that dietary creatine rapidly and specifically represses formation of arginine-glycine transamidinase, the first enzyme of the biosynthetic pathway leading exclusively to creatine (cf. Biochim. Biophys. Acta 36:575, 1959). Related compounds are not as effective, nor is formation of the other enzyme involved in creatine biosynthesis, guanidinoacetate methylferase, repressed. Superimposed upon this feedback control of transamidinase synthesis by the biosynthetic end-product, creatine, is an even more subtle hormonal control. We have observed that, although pre-pubertal male and female rats have the same levels of kidney transamidinase, the concentration of kidney transamidinase in adult male rats is twice that of adult females. It is thus possible that the different creatinine excretion coefficients of men and women may result from differences in transamidinase levels. (Supported by a Senior Research Fellowship from the Public Health Service).

5 *Biochemistry*

FEEDBACK CONTROL OF THE ACTION OF ISOCITRATASE IN ESCHERICHIA COLI. H. E. Umbarger, Dept. of Bacteriology and Immunology, Harvard Medical School, Boston, Mass.

Two enzymes, isocitratase and malate synthetase, provide for the generation of the dicarboxylic acids required during growth of Pseudomonas species and E. coli on acetate. H. Kornberg (Nature, 182, 1431 (1958)) has demonstrated in Ps. ovalis that the synthesis of isocitratase is repressed by the addition of succinate to an acetate-mineral salts medium. A similar repression has now been demonstrated in E. coli. Furthermore, in extracts the action of isocitratase is strongly inhibited by oxalacetate but not by malate, fumarate, aspartate or pyruvate. It is proposed that this inhibition is a negative feedback mechanism maintaining the dicarboxylic acids at a level that assures continuous functionning of the citric acid cycle. In these experiments, isocitratase activity was determined by measuring isocitrate formed from succinate and glyoxylate. Isocitrate was measured by means of isocitric dehydrogenase and triphospho-3-acetylpyridine nucleotide (APTPN). APTPN is reduced by isocitratase, but APTPNH, unlike TPNH, is not oxidised by the glyoxylic reductase present in E. coli extracts.

6 *Pharmacology*

TACHYPHYLAXIS AS A MECHANISM FOR THE REGULATION OF RESPONSES TO AUTONOMIC MEDIATORS. Efraín G. Pardo, José Luis Magaña* and Roberto Vargas*. Dept. of Pharmacology, Escuela Nacional de Medicina, México, D. F.

It has been proposed that tachyphylaxis may be defined as the situation in which the response to a given concentration of active drug obtained by the gradual accumulation on repeated administration is smaller that the response to the same concentration obtained after a single dose, and that it may result from the possibly shorter duration of the local disturbance created by the formation of the drug-receptor combination as compared to the half-life of this combination. This would imply that the phenomenon will be more readily observed in the case of those compounds which dissociate slowly from their union at the receptor site, but that it should also be expected in the case of the normal mediators in the autonomic nervous system. Suitably designed experiments on diverse systems indicate that this is the case and provide evidence for the above mentioned interpretation of the phenomenon.

1 *Physiology*

RESPIRATION OF EPIDERMIS AND DERMIS FROM NORMAL AND HYPOTHYROID RATS. A. Kurt Weiss and Margaret A. Hersch.* Dept. of Physiology, Univ. of Miami School of Med., Coral Gables, Fla.

A sample of skin from the previously depilated ventral surface of a Holtzman rat can be sliced with a Stadie-Riggs microtome so that the first slice contains epidermis as well as dermis, and the next slice contains dermis only. After termination of the oxygen consumption measurements of both slices individually by the direct Warburg method, further incubation of the first slice in a buffered 2 N NaI solution permits separation of the epidermis from the dermis. After determining the nitrogen contents of the epidermis and the dermis slices, their oxygen consumption rates can be computed. These calculations show that the Q_{O_2} (Prot.) of the epidermis of normal rats is 56, and some 40 times greater than that of the dermis. In a group of severely hypothyroid rats the Q_{O_2} of the epidermis was reduced to a value of 16. The oxygen consumption of the dermis appeared to be slightly reduced in the hypothyroid rat, but the change was not statistically significant. Since dermis is a connective tissue the effect of hypothyroidism on other connective tissues was also investigated. Lung tissue, as well as connective tissue which developed in subcutaneous implants of Ivalon sponges, did not show significantly different Q_{O_2} values in hypothyroid animals when compared with controls. (Supported by grant RG-5100 from the NIH.)

2 *Physiology*

ADRENERGIC SUBSTANCES OF ADRENAL AND OF NON-ADRENAL ORIGIN. Irene E. Loewenfeld, Columbia, New York. (Motion Picture.)

It is generally known that sensory and emotional stimulation of intact, awake animals — or hypothalamic stimulation in anaesthetized animals — cause 1) an increase of adrenaline from the adrenal medulla and 2) a rise in the blood level of nor-adrenaline. The latter effect, brought about mainly by liberation of nor-adrenaline at sympathetic nerve endings, is generally believed to be of minor physiological importance. Using the denervated dilatator pupillae of the cat as indicator, it was found that in response to moderate psycho-sensory stimulation of awake cats, or to hypothalamic stimulation in narcotized cats, adrenergic substances carried by the blood appear in the eye within 2-3 seconds after the beginning of stimulation; the reaction declines within 7-10 seconds. Since adrenal adrenaline, and adrenergic substances from any other source discharging into veins, require about 10-15 seconds to reach the eye, it is concluded that the humoral reaction to moderate sensory, emotional or hypothalamic stimulation is caused by adrenergic substances poured into the arterial circulation by the heart or the great arteries. Adrenal adrenaline, in sufficient quantities to affect the denervated iris, is mobilized only after prolonged or intense stimulation. (Supported by the Harriman Fund and by a grant from the Public Health Service (B-253).

3 *Physiology*

HORMONAL REPLACEMENT OF PHOTOPERIOD AS A STIMULUS FOR GROWTH IN A LIZARD. Anthony Di Maggio III (intr. by L. A. Toth.) Louisiana State University School of Medicine, New Orleans, La.

Many of the seasonal behavioral and metabolic changes that occur in the lizard, Anolis carolinensis can be altered or mimicked through control of the photoperiod. Exposure to 18 hrs. of light/24 hrs. stimulates growth; exposure to 9 hrs. of light/24 hrs. depresses growth (Fox and Dessauer. J. Exp. Zool. 134:557, 1957). The table below summarizes the effect of i. p. injections of 3 hormones on growth of juvenile lizards exposed to only 6 hrs. of light/24 hrs. for a 2 month period.

GROUP	DOSAGE	WEIGHT GAIN (% ± S. D.)
Controls	0.2 ml. H_2O/day	9.6 ± 8.6
Growth hormone(G)*	10 mg/kg/day	22.6 ± 12.7
Desiccated thyroid(T)	7 mg/kg/day	16.0 ± 10.0
Insulin (I)	500 U/kg/day	5.4 ± 8.4
G. + T. + I.	as above	29.8 ± 17.6

Growth hormone and G. + T. + I., like long photoperiods, are effective in stimulating growth in Anolis.

* Gift of Endocrinology Study Section of N. I. H.

4 *Physiology*

ACTIVATION OF A COORDINATED RESPONSE IN HYDRA BY REDUCED GLUTATHIONE OR PROTEASES. Howard M. Lenhoff (intr. by E. L. Chambers.) Howard Hughes Medical Institute, Miami, Florida.

The mechanism by which excitatory compounds act on cells is not understood. As one approach to this problem we are studying the action of reduced glutathione (GSH) in eliciting the coordinated feeding reflex of Hydra. The reflex was measured quantitatively by averaging the time that the mouths of Hydra remained open in the presence of GSH. The normal response was 22 min. The Hydra responded maximally 1-2 days following a previous exposure to GSH. The plotting of GSH concentration against the reflex time gave a typical saturation curve. The maximal and half maximal responses occurred at 10^{-5} M and 2 x 10^{-6} M GSH respectively. Ca^{++} at 10^{-4} M was required for the action of GSH; only Sr^{++} partially replaced Ca^{++}. Versene and Mg^{++} were inhibitory. The inhibitory effects of these agents were reversed by Ca^{++}. The anions present also affected the response with $Cl^- > Br^- > I^- = NO_3^-$. The reflex was elicited in the absence of GSH by the enzymic action of proteases. Dialyzed papain and ficin did not act until cysteine was added; the boiled enzymes were without effect. The activation by trypsin was stopped by soybean trypsin-inhibitor. The reflex was not elicited by 18 purified enzymes that were not proteases. This experimental system may provide general information about the mechanism of action of excitatory compounds.

5 *Physiology*

INTERACTION OF EFFECTS OF PINEALECTOMY AND CONSTANT LIGHT IN THE FEMALE RAT. R. J. Wurtman, W. Roth, M. D. Altschule and J. J. Wurtman (intr. by R. O. Greep). Harvard University, Boston, Massachusetts.

Fifty 25-day old female rats were unoperated, sham-operated, or pinealectomized, and maintained in constant light or darkness for 80 days, after which they were weighed and autopsied. At 80 days, light and darkness were without effect on the ovarian, adrenal and uterine weights of unoperated animals; this is in agreement with previous publications showing that 56 days of light cause ovarian hypertrophy, while 79 days of light or darkness generate ovaries of equal weight. Light was associated, however, with a decrease ($P < .05$) in pineal weight. Among operated animals, either pinealectomy or exposure to constant light, or both, resulted in ovarian and adrenal hypertrophy, but the effects of the two procedures were not additive. Two populations of animals were created, which differed significantly in ovarian weight ($P < .01$) and adrenal weight ($P < .001$): (1) sham-operated animals kept in the dark, and (2) all other operated animals. This suggests that both pineal and light influences on ovarian and adrenal weight operate by the same pathway. Pinealectomy resulted in no change in uterine weight, but the uteri of operated animals kept in the dark weighed significantly less ($P < .001$) than those of animals kept in the light.

6 *Biochemistry*

ESTROGEN STIMULATION OF ATP PRODUCTION AND PROTEIN SYNTHESIS. C.A. Villee, P.B. Joel*, J.M. Loring*, and J.M. Spencer*, Dept. of Biological Chemistry, Harvard Medical School, Boston.

Human placenta and certain other tissues contain an estrogen stimulable pyridine nucleotide transhydrogenase (J.B.C. 233, 42,1958). Placental homogenates incubated with citrate and inorganic P^{32} respond to added estradiol with marked increases in rates of oxygen consumption, citrate utilization, ketoglutarate production and of incorporation of P^{32} into the terminal phosphate of ATP. With placental slices, estrogen increases the rate of incorporation of labelled carbon from glycine-1-C^{14}, acetate-2-C^{14}, or pyruvate-2-C^{14} into tissue protein and fatty acids, and into the purine bases of the tissue nucleic acids. These results support the hypothesis that estrogen stimulation of the transhydrogenase increases production of biologically useful energy and secondarily increases rates of synthesis of proteins, fats, and nucleic acids. Pyridine nucleotides in placenta were measured using DPN specific alcohol dehydrogenase and TPN specific glucose-6-phosphate dehydrogenase coupled with diaphorase and dichlorophenolindophenol. Fresh placenta contains .21,.04,.005,and .009 μmoles per g tissue of DPN, DPNH, TPN, and TPNH respectively. Thus, placenta contains DPN largely in the oxidized state and TPN in the reduced state, which favors the transfer of hydrogen from TPNH to DPN by the pyridine nucleotide transhydrogenase.

7 *Biochemistry*

STEROID HORMONE EFFECTS ON LIVER ALDEHYDE DEHYDROGENASE ACTIVITY. Elizabeth S. Maxwell and Yale J. Topper. Natl. Insts. of Health, Bethesda, Md.

The DPN linked aldehyde dehydrogenase activity in a 40-60% saturated $(NH_4)_2SO_4$ precipitate from the soluble portion of rabbit liver is inhibited by 10^{-5} M progesterone, testosterone and deoxycorticosterone, stimulated by 10^{-4} M cortisone, and relatively unaffected by other closely related steroids. Progesterone (10^{-5} M) decreased the rate of DPN reduction 25%, 35%, 66% and 72% with butylaldehyde, propionaldehyde, DL-glyceraldehyde and glycolaldehyde, respectively. In the latter case progesterone was 60 times more effective in reducing the level of DPNH than would be predicted if it were acting stoichiometrically as an acceptor of electrons from reduced pyridine nucleotide. Cortisone (10^{-4} M) increased the rate about 50% with glycolaldehyde and propionaldehyde. Whether these effects are due to direct action of steroids on aldehyde dehydrogenase or are the result of coupled reactions is not yet certain. Progesterone has little effect on purified calf liver aldehyde dehydrogenase or on aldehyde dehydrogenase activity in a 0-40% $(NH_4)_2SO_4$ precipitate from rabbit liver. An indirect effect of the steroid sensitive activity on the oxidation of galactose has been reported (Topper, et al. Biochim. et Biophys. Acta (in press.)

8 *Biochemistry*

STIMULATION OF LYMPHOSARCOMA ATPase ACTIVITY BY STEROIDS. Melvin Blecher (intr. by H. Strecker) Albert Einstein Coll. of Med., Yeshiva Univ., New York.

Previously reported (J. Biol. Chem., 235 (1960)) inhibitions by cortisol (F) and deoxycorticosterone (DOC) of anaerobic glycolysis of glucose and hexose monophosphates by homogenates of rat lymphosarcoma (Murphy-Sturm) were ascribed, in part, to steroidal augmentation of ATPase activities. Direct evidence has now been obtained for a marked in vitro stimulatory action of DOC and F on Mg^{++} -activated ATPase activity of freshly prepared lymphosarcoma mitochondria and whole homogenates; no steroid effect was observed in microsomal or soluble cytoplasmic fractions. Ageing of mitochondria or homogenates in 0.35M sucrose (1 hr, 30°) released latent ATPase activity; no steroid effect was then evident. Stimulation of mitochondrial ATPase activity by DOC and F was proportional to steroid concentration (0.1 to 0.6 mM), and uninfluenced by gas phase (air or N_2) or presence or absence of serum albumin (BSA) in the medium; BSA, however, was required to keep ATPase activity latent. Specificity studies revealed paralleling relationships between steroid structure and inhibitory influences on glucose metabolism by lymphoid tissue (Recent Progr. Hormone Res., 15, 391 (1959)) and degree of stimulation of lymphosarcoma mitochondrial ATPase activity. (Supported by grants from American Cancer Soc. and Natl. Cancer Inst.)

9 *Biochemistry*

THE PROGESTERONE-LIKE EFFECTS OF MENTHOL ON GALACTOSE METABOLISM. T. D. Elder*, S. Segal*, E. S. Maxwell and Y. J. Topper. National Institutes of Health, Bethesda, Md.

It was reported that progesterone stimulates the conversion of galactose-1-C^{14} to $C^{14}O_2$ by liver slices and homogenates by indirectly stimulating the UDPGal-4-epimerase reaction. The mechanism of this effect was at least partially ascribed to a lowering of the DPNH level consequent to inhibition by the hormone of certain aldehyde dehydrogenase fractions. We also reported that progesterone enabled 3 prepubertal subjects with congenital galactosemia to metabolize a substantial fraction of a tracer dose of galactose-1-C^{14} to $C^{14}O_2$. It has been observed that all of these effects can be produced by menthol: 1) Only liver and small intestine slices, of all rabbit tissues tested, show increased capacity to oxidize galactose-1-C^{14} to $C^{14}O_2$ in the presence of menthol when alcoholic substrates are added; 2) UDPGal-1-C^{14} metabolism by the soluble fraction of liver homogenates, but not that of UDPG-1-C^{14}, is enhanced by this terpene; 3) some aldehyde dehydrogenase fractions derived from liver are inhibited by menthol and/or menthone; 4) the capacity of 2 prepubertal congenitally galactosemic subjects to convert a tracer dose of galactose-1-C^{14} to $C^{14}O_2$ was greatly increased after the oral administration of menthol. It is not known whether the mechanism of this in vivo effect is the same as that observed in vitro.

XUM

/0 *Biochemistry*

INTERACTION OF STEROIDS WITH UREA AND RELATED COMPOUNDS. Gene F. Lata and Le Kim Dac (intr. by G. Kalnitsky.) Dept. of Biochem., State Univ. of Iowa, Iowa City, Iowa.

Interactions of various Δ^4-3keto-steroids with aqueous urea, acetamide, formamide, N-alkyl acetamides N-acetyl-N'-methyl urea, and 2,5-diketopiperazine were studied by solvent partition and by incubation of solid steroid. Steroid determinations performed spectrophotometrically. Testosterone esters showed no interaction. Testosterone, epi-testosterone, 17α-methyl testosterone and Δ^4-androstene-3,17-dione were solubilized by urea to decreasing extents as listed. Increased solubilization by increasing urea concentrations (0 to 8 molar urea) was greater at 10°C than at 25°C or 40°C. Formamide, acetamide and N-alkyl acetamides were as effective as urea in solubilizing testosterone. 2,5-Diketopiperazine (0.03 to 0.08 molar) solubilized testosterone, but not testosterone esters, Δ^4-androstene-3,17-dione or, 17α-methyl testosterone. N-Acetyl-N'-methyl urea, dihydrouracil and uracil were about ten-fold more effective than urea in solubilizing testosterone. For urea interaction a free 17-hydroxyl group either α- or β- is necessary, although 17-ketones also interact. In urea systems neither λ_{max} (ca. 249 mμ) nor A_m for Δ^4-3-keto group were altered. Results with diketopiperazine suggest the importance of a 17β configuration for steroid interaction with this planar compound. (Supported by Grants RG 4741; A-3079 U.S.P.H.S.)

/1 *Pharmacology*

DIFFERENCE IN METABOLIC RESPONSE BETWEEN NORMAL AND HYPERTHYROID LIVER SLICES TO A CHANGE IN SURROUNDING MEDIUM. M.A. Spirtes and Betsy Eaton*. Hahnemann Medical College, Philadelphia, Pa.

It was reported previously that the low glycolytic rate of rat liver slices incubated in a glucose-Ringer solution anaerobically or aerobically increased seven to tenfold when the surrounding medium was changed to a hypertonic sucrose solution plus a number of cofactors. The reaction of liver slices from hyperthyroid rats to the same sucrose medium will be discussed. Wistar rats were injected with one milligram of l-thyroxin daily for four days following which controls and the thyroxinized rats were beheaded and liver slices prepared with a double-edged razor blade. The slices were kept in cold 0.9% NaCl until placed in Warburg vessels with medium at 37°C. aerobically or anerobically. The control liver slice glycolysis measured as lactate produced rose from four gamma per mgm. dry weight per hour in the presence of Ringer-glucose solution to 33.9 gamma when the reinforced sucrose medium was added with glucose as substrate. The hyperthyroid liver slices produced only 2.5 gamma lactate in the sucrose-enriched medium either anerobically or aerobically. Possible interpretations of this finding will be discussed.

027. BIOLOGICAL REGULATION–Rhythms

/ *Physiology*

CIRCADIAN PERFORMANCE RHYTHMS IN MEN ADAPTING TO AN 8-HR. DAY. G. T. Hautr*, G. B. Steinkamp*, W. R. Hawkins*, and F. Halberg School of Aviation Med. Brooks AFB, Tex. and U. of Minn. Mpls.

Trained individuals (5) were singly confined in a sealed 50-cubic foot chamber (P_B and P_{IO_2} at 380 mm. and 150 mm. Hg., respectively; P_{ICO_2} from 4.25 to 27.5 mm. Hg.). Temperature and humidity varied, but not 24-hr. periodically; level of illumination constantly high; no visual contact with outside. Constant internal noise, ~ 85 db., effectively masked external sounds. For 7 days, subject was committed to alternate 4-hr. periods of rest and work (provided by complex operator system). Subjects had wristwatches set to local time but "thought mostly" in terms of 4-on/4-off /synchronizer interaction (Halberg, et al., Pub. 55 AAAS, 1959, p. 836)/. Different physiologic and psychologic functions of a given subject adjusted to a varying extent to 8-hr. day, extent of adaptations differing also among subjects. Autocorrelograms uncovered persisting circadian periodicity (average periods of ~ 24 hrs., not necessarily of exactly that length) in heart and respiration rates and in performance. Circadian components were more significant when adaptation to 8-hr. day was more difficult. Adaptations to artificial schedules depend upon extent of modifiability of circadian physiologic periodicity shown herein to operate in subjects living for one week on a 4-on/4-off schedule.

/2 *Physiology*

BEHAVIORAL EFFECTS OF RANDOM TEMPERATURE CYCLES IN LIZARDS. J. Langdon Taylor, Jr.* and Robert D. Tschirgi. U. of California, Los Angeles, California.

Activity of isolated adult Sceloporus magister lizards under conditions of constant sound level in a homogeneously illuminated circular chamber, has been studied continuously for periods up to 60 days by means of time lapse photography. Lizards subjected to regular 24 hour environmental temperature cycles (20°-35°C) quickly adapt to these conditions, show stable rhythmic behavior patterns, bury under the sand as temperature falls and emerge after temperature again rises. After several days they even "anticipate" the drop in temperature by burying a short time before the actual cold phase begins. This 24 hour "cold response" persists for up to 7 days under conditions of constant warm temperature at the times corresponding to the previous cold phase of the cycle. A lizard, exposed to random durations at the same two temperatures, followed the variations for the first few cycles but then stopped burying activity, became almost immobile, and would not eat or drink. When removed from the chamber and replaced in the terrarium in which it had previously lived for 2 months, it remained unresponsive to all but the most intense stimuli and died within 4 days. This environmental trigger of rhythmic behavior (temperature changes) when presented aperiodically appears sufficient to induce lethal alterations in behavior.

028. BLOOD

/ *Physiology*

THE EFFECT OF BUFFERING ACD BLOOD WITH THAM ON THE SURVIVAL OF DOGS TRANSFUSED AFTER MASSIVE HEMORRHAGE. Gabriel G.Nahas, Arnold Mittelman* and William M. Manger. Depts. of Anesthesiology, Surgery and Medicine, Coll. of Physicians & Surgeons, Columbia Univ., New York, N. Y.

A volume of 49 ml/kg of blood was withdrawn in approximately 10 minutes from the femoral artery of 16 healthy mongrel dogs until they presented marked alterations of ECG and minimal pulse pressure. Arterial pH was 7.36 at the end of bleeding. Eight animals were transfused in 4-12 minutes with an equal amount of homologous blood stored at 4°C for 1-10 days in ACD solution and whose average pH was 6.47 (6.34-6.91). Seven of these dogs died in cardiac arrest during the transfusion, and the arterial pH at arrest was 6.92. Eight other animals were treated in a similar fashion except that 12-15 mM of trishydroxymethylaminomethane (THAM) in a .3 to .6 M solution was added to the transfused blood to raise the pH to 7.56 (7.410-7.800). Seven of these dogs survived and average arterial pH at the end of transfusion was 7.20. These experiments indicate that massive blood replacement with ACD stored blood (pH 6.40-6.70) may not be harmless.

/2 *Physiology*

SOME PROPERTIES OF PLATELET COFACTOR I (FACTOR VIII). William J. Baker * and Walter H. Seegers. Dept. Physiology and Pharmacology, Wayne State University, Detroit, Michigan.

Platelet cofactor I preparations from bovine plasma were studied from the viewpoint of stability in the presence of chemicals selected strategically. The preparations were of high quality having about 86 per cent of the protein as one component by ultracentrifuge analysis. The activity was easily destroyed with hydrogen peroxide, sodium sulfite, phenylhydrazine, formalin, and acetone, and by the process of freeze drying. The activity was not lost in the presence of oxygen, ascorbic acid, cysteine, merthiolate, parachloromercuribenzoate or 0.5 per cent phenol. We use the latter as a preservative. In the native plasma the activity is not adsorbed on barium carbonate, barium sulfate, or magnesium hydroxide. Nor is it destroyed by ether; but, in purified form it is adsorbed on these adsorbants and is destroyed by ether. In the purified preparations the activity is not adsorbed on IRC-50 resin thus making it possible to use this adsorbant to remove any thrombin that might be added to a platelet cofactor I preparation. By this technic it was possible to show that thrombin alone does not easily destroy platelet cofactor I activity. (Supported by USPHS Grant H-2026 NIH).

3 *Biochemistry*

SELECTIVE ALTERATION OF LIVER MITOCHONDRIA CAUSED BY HEPATIC CARCINOGENS. J.C.Arcos, D.Zickafoose and R.D.Warren (intr. by G.K.Davis) Cancer Research Laboratory, University of Florida, Gainesville, Florida.

The correlation between tumor incidence and changes in microsomal and mitochondrial swelling (Arcos et al., Experientia, 15: 316, 1959; J. Biophys. Biochem. Cytol., in press) during feeding of 3'-methyl-4-dimethylaminoazobenzene (3'-Me-DAB) to rats has now been extended to other hepatic carcinogens: 4'-fluoro-DAB, 4'-ethyl-2-methyl-DAB (both at 2.40mM/kg) and DL-ethionine (19mM/kg) were fed in a semi-synthetic diet and 2-acetylaminofluorene (1.62mM/kg) in an enriched chow diet; tannic acid was given subcutaneously (150mg/kg) twice weekly. All these produced alterations of mitochondrial swelling similar to those found with 3'-Me-DAB. In contrast to these, feeding of the non-carcinogenic azobenzene and 4-diethylaminoazobenzene did not affect swelling, which is consistent with results found previously with 2-Me-DAB (loc.cit.). Feeding of 3'-Me-DAB (4.80mM/kg) in the chow diet to the resistant guinea pig did not affect swelling in the thyroxine, $HgCl_2$ and $CaCl_2$ test systems. (Supported by the U.S.P.H.S. Grant C-4251).

4 *Biochemistry*

SPECTROPHOTOMETRIC MEASUREMENT OF BLOOD pH. F. Lee Rodkey, Division of Chemistry, Naval Medical Research Institute, Bethesda, Md.

Phenol red is used in a number of spectrophotometric and colorimetric methods for blood pH. Each method requires special correction factors when applied to blood of various species as well as preparation and maintenance of many reference solutions. The present method requires no standard solutions and may be applied to blood samples from several species. Blood, plasma, or serum is diluted 1:21 with an adjusted, isotonic indicator-diluent solution containing NaCl, NaF, phenol red, and sodium naphthalene-2-sulfonate. Absorbancy is measured at 550 mμ with the temperature of the solution at 37°. Reference measurements are made on a solution containing the same concentration of phenol red in the completely alkaline form and on a diluted sample without indicator. These data are used with the known pK' of phenol red to calculate the pH of the diluted sample. Duplicate measurements are obtained with 0.6 ml of blood or plasma. Incorporation of the non-buffering sulfonate into the diluent solution completely inhibits binding of phenol red by serum proteins in all species studied. The pH values of the undiluted samples have been measured by glass electrode and compared with results obtained by the spectrophotometric method.

5 *Biochemistry*

STUDIES OF UNBOUND AMINO ACID DISTRIBUTION IN PLASMA, ERYTHROCYTES AND LEUKOCYTES OF NORMAL HUMAN SUBJECTS. R. H. McMenamy (intr. by J. L. Oncley.) Harvard Medical School, Boston, Mass.

The relationship in the concentration of unbound amino acids in the plasma and cells of human subjects is of considerable interest from both the viewpoint of normal cell function and its alteration under conditions of disease or stress. This presentation reports the distribution of unbound amino acids and other small molecules between the plasma, erythrocytes and leukocytes of normal human fasting subjects. Determination of the amino acids is made by chromatographic analyses using salt saturated systems. (McMenamy, R. H., Lund, C. C. and Oncley, J. L., J. Clin. Invest., 36, 1672 (1957) Methods of obtaining leukocytes and erythrocytes for subsequent quantitative analyses of the unbound amino acids are described. Many of the amino acids in the erythrocytes were found to be equal in concentration with those in the plasma. In the leukocytes the concentration of individual amino acids was 4.8 to approximately 100-fold higher than in the plasma. (Supported by a grant from the National Institutes of Health.)

6 *Pharmacology*

SEPARATION OF FACTORS VII, IX AND X FROM PROTHROMBIN AND THEIR ACTION IN PROTHROMBIN CONVERSION. Robert Goldstein (intr. by J.P. Bunker.) New Eng. Cent. Hosp. and Tufts Univ. School of Med., Boston, Mass.

Human and bovine prothrombin fractions of high specific activity (10-14000 units/mg. N) prepared by usual techniques still exhibit high activity of factors VII, IX and X. When such fractions were subjected to continuous flow electrophoresis there were differences in the migration of the coagulation factors. From human material the following fractions were obtained: 1) factor VII devoid of the other factors; 2) prothrombin low in VII, IX and X activity; 3) IX and X devoid of prothrombin and VII. With bovine material VII and prothrombin migrated together and were completely separated from IX and X which migrated together. Before electrophoresis prothrombin fractions were readily converted to thrombin by: 1) tissue thromboplastin, factor V and Ca; 2) 25% citrate; 3) platelets, $BaSO_4$ adsorbed plasma and Ca. Prothrombin obtained from electrophoresis was not readily converted by any of these systems unless the non-prothrombin fractions were added. For optimal conversion with thromboplastin factors VII and X were necessary; for conversion by 25% citrate, IX and perhaps X were essential; and for conversion by platelets, IX and X were essential. (Supported by PHS Grant H3649.)

7 *Pharmacology*

THE COMPLEX PATTERN OF RESPONSE TO COUMARIN DRUG THERAPY IN HUMANS. S. Gollub, A. W. Ulin and W. Likoff (intr. by N. Back.) Blood Coag. Research Lab., Hahnemann Hosp., Phila., Pa.

Coumarins have been shown to affect other coag. proteins than prothrombin: factors 9, 7 and perhaps more. To assess the clinical significance of these findings, 12 normal persons and over 40 patients on acute and chronic anticoagulant therapy were serially studied. Stuart and 7 were found to be immediately depressed and maintained lower than the one stage prothrombin level for most patients. TGT showed opposite tendencies. However, some patients had different patterns of response. Marked and unexpected variation in TGT occurred during relative stability of one stage prothrombin values, in some cases. Stuart factor depression may have been correlated with hematuria in two instances. The one stage prothrombin time appears to be inadequate as a test for reflecting the total and variable anticoagulant action of coumarin drug therapy. (Supported by a grant from S. E. Penna. Heart Assn.)

8 *Pharmacology*

IN VIVO FORMATION OF A VASODILATOR SUBSTANCE (PLASMA KININ) BY PLASMIN. N. Back. Roswell Park Memorial Inst. and Univ. of Buffalo Grad. School, Buffalo, New York.

Plasmin, incubated in vitro with plasma globulins, forms a vasodilator substance indistinguishable from bradykinin (Lewis; J. Physiol. 140, 285, 1958). Rapid intravenous injection of streptokinase-, uorkinase-, or spontaneously-activated plasmin in dogs results in a transient hypotension which is abolished after successive administrations (Back; Pharmacologist 1, 56, 1959). Plasmin hypotension did not occur in dogs rendered tachyphylactic to the hypotensive effect of human urinary kallikrein, an enzyme causing the formation of a vasodilator polypeptide kallidin (considered to be identical with bradykinin). Animals tachyphylactic to human plasmin were unresponsive to hypotensive doses of kallikrein. This cross-tachyphylaxis was not observed with other plasma proteins. Supernates obtained by alcohol precipitation of plasminogen and plasmin at various time intervals during activation with streptokinase contained no hypotensive substances. It appears that hypotension resulting from rapid injection of large doses of plasmin is due to release of a plasma kinin, similar to, or identical with kallidin. Tachyphylaxis to plasma may be due to depletion of kallikreininogen and kallidinogen. Kallikrein may be hypotensive per se in addition to forming kallidin. (Supported by a grant from Public Health Service).

9 *Pathology*

LOCALIZATION OF RUSSELL-BODY FORMATION IN THE HUMAN PLASMA CELL. Ronald A. Welsh (intr. by Russell L. Holman) Louisiana State Univ. School of Med. New Orleans, La.

Russell bodies, found intracellularly in plasma cells, have been shown in work by others to be a mucoprotein, positive for gamma globulin by fluorescent technics, but little is known of their exact localization within the cell or the possible intracellular mechanisms involved. Human gastric mucosa obtained at surgery was fixed in buffered osmium tetroxide, embedded in methacrylate, and ultrathin sections were studied by electron microscopy and light microscopy. Russell bodies were found as dense oval bodies, always within the intracisternal space of the endoplasmic reticulum. The nucleus, mitochondria, and other morphological structures appeared normal, thus tending to negate a degenerative process. The suggestion is offered that the Russell body results from a condensation of intracisternal secretion.

10 *Immunology*

IMMUNE ACTIVATION OF A CYTOLYTIC FACTOR IN SERUM; ITS PREPARATION AND PROPERTIES. Bertil Björklund (intr. by Maurice Landy.) Immunological Research Laboratory, State Bact. Lab. and Radiumhemmet, Stockholm, Sweden.

A lytic system present in normal serum and operating independently of complement has been identified. This system is present in serum as an inactive complex consisting of a cytolytic factor (CF) coupled with an inhibitor (CFI). Both factors have been prepared in a relatively pure state from human and from horse serum. In the presence of normal human serum, horse antibody to a human carcinoma antigen reacts with HeLa cells or other established cells of human origin and causes the release of CF with consequent fragmentation of the cells. CF was prepared by dialysis of serum against M/150 phosphate buffer pH 7.9, followed by separation in a Spinco Model CP continuous curtain electrophoresis apparatus. Details of the preparative method will be presented. The migration of CF is equal to or faster than that of albumin. In the isolated state it withstands one half an hour at 71° C. but not 73° C. It is readily adsorbed on kaolin, bentonite, PVC, CMC, DEAE, and calcium phosphate gel. CF possesses esterase activity and causes fragmentation of the cell membrane of a number of established strains of mammalian cells.

11 *Immunology*

THE VALUE OF ACRIDINE ORANGE FOR STERILIZING VIRUSES IN PLASMA AND WHOLE BLOOD. Gerald LoGrippo and Daniel H. Basinski. Dept. of Labs., Henry Ford Hospital, Detroit, Mich.

The combined chemical-physical action of acridine orange (AO) and ultraviolet (U.V.) irradiation is an effective mechanism for inactivation of certain virus agents in plasma. The affinity of acridine orange for nucleoprotein and the excitation of this agent with U.V. are demonstrated upon E. coli T-3 phage seeded in plasma. This mechanism shows a more specific virucidal action against the infective nucleoprotein component of the virus and less damaging effect upon the plasma protein components. This is shown by the preservation of the coagulation components and by electrophoretic patterns of plasma at the virucidal concentrations of the combined procedure. Acridine orange in concentrations of 10 mg/l and 1 mg/l sterilizes plasma seeded with 2×10^8 phage particles per ml. of plasma in 24 and 72 hours respectively when stored at 4° C. The synergistic action of AO with U.V. sterilizes when only 0.1 mg/l of AO is combined with U.V. (3.6 mw/sec/cm^2.) These concentrations alone are not sterilizing. Poliomyelitis and Coxsackie viruses have not been inactivated by this procedure. Sterilization of whole blood presents different problems.

029. BLOOD–Bone Marrow

1 *Nutrition*

FOLIC ACID REQUIREMENTS IN MAN. Sheehy, T.W., Rubini, M.R., Sigler, M.H., Perez-Santiago, E., Baco-Dapena, R., and Santini, R. (intr. John B. Youmans.) U.S.A. Tropical Research Medical Laboratory and University of Puerto Rico School of Medicine, San Juan, Puerto Rico.

Man's minimum requirement for folic acid (FA) was estimated in tropical sprue, a disease associated with apparent FA deficiency, by defining the minimum dose capable of inducing a hematologic response. In six patients with megaloblastic anemia, prompt hematologic and general recovery followed oral administration of 25 ucg FA daily (1/600 the usual therapeutic dose). This quantity was insufficient for repletion or storage, as megaloblastic changes recurred in the bone marrow when medication was stopped briefly, or erythropoiesis was accelerated by repeated phlebotomies. Microbiologic assay (Strep. fecalis) showed the patients' diet provided 1000 to 1300 ucg/day FA-like activity, of which 30 to 50 ucg were "free". Intestinal fluid from patients with sprue was capable of splitting the dietary FA complexes in vitro, yet these dietary analogues failed to correct the anemia. Although synthetic FA is absorbed better than certain conjugates, the minute dose capable of inducing hematologic remission in tropical sprue suggests that the daily FA requirement for normoblastic maturation is less than 100 ucg.

030. BLOOD–Coagulation

1 *Physiology*

THE WETTABILITY OF GLASS AND FIBRINIZED SURFACES IN RELATION TO INTRAVASCULAR BLOOD COAGULATION. A.L. Copley and G.W.Scott Blair*. N.Y.Med.Coll., New York; Royal Coll.Surgeons of England, London and Natl.Inst. Res.in Dairying, Univ.Reading, England.

A fibrin surface is supposed to constitute the inner or endoendothelial lining of living blood vessels. In extra-corporal studies of blood contacting a fibrin surface, fibrinization decreases apparent viscosity and wall adherence, has an anticoagulant action and enhances clot retraction. (See: Flow Properties of Blood and Other Biological Systems, ed. Copley & Stainsby, Pergamon, London, 1960). The wettability of fibrin surfaces was therefore measured. The rise of liquids into a vertical capillary tube depends on tube radius, surface tension of the liquid to air and the wetting of the tube's surface by the liquid. Keeping the first 2 factors constant, in initially dry tubes, glass wets more readily than a fibrin surface, though wet fibrin shows less interfacial tension than wet glass, with water or plasma. These expts. amplify the above earlier findings, suggesting that blood coagulation, intravascular or extra-corporal, is not concerned with the wettability of the fibrin lining. (Aided in part by grant H-4793 from Natl. Insts. Health, Bethesda, Md.)

2 *Physiology*

COAGULATION DEFECTS AND PULMONARY HYPERTENSION EXPERIMENTALLY PRODUCED BY PULMONARY EMBOLIZATION WITH INFUSIONS OF PARTICULATE MATTER. J. W. Hampton*, W. E. Jaques, R. M. Bird, and D. M. Selby*. Univ. of Okla. Med. Center, Okla. City, Okla.

The infusion of amniotic fluid into dogs produces a fall in fibrinogen with a defect functionally similar to plasma thromboplastin antecedent deficiency; pulmonary hypertension; and multiple pulmonary thrombi with subsequent intimal thickening (Federation Proc., 18, 64 and 483, 1959). Current studies indicate that these changes are the nonspecific result of multiple pulmonary emboli. Fractionation of amniotic fluid by centrifugation at 4000 r.p.m. for 2 hrs. did not separate all particulate matter and did not yield an inactive fraction when infused. The supernatant gave changes appearing later and of smaller magnitude than whole fluid or sediment. Seitz filtered fluid was essentially inactive. Prior infusion of human fibrinolysin blocked the hypertensive response usually evoked by whole amniotic fluid but only delayed the clotting defects. Pulmonary embolization with glass beads (450 $\pm$ microns in diameter) produced coagulation defects and pulmonary hypertension as readily as did whole amniotic fluid infusion. The late morphological findings were similar in all experiments in which particulate matter was infused.

3 **Physiology**

INDUCED "HYPERPROTHROMBINEMIA" IN GUINEA PIGS. Murray Weiner and Peter G. Dayton* N.Y.U. Res. Serv. Goldwater Mem. Hosp., N.Y., and Nat. Heart Inst., Bethesda, Md.

Although numerous agents induce hypoprothrombinemia, no drug has been reported to induce a sustained hyperprothrombinemia. We found that single 100-200 mg/kg intraperitoneal doses of 4-aminoantipyrine to normal guinea pigs were able to reduce the one stage prothrombin time (Simplastin) from 34 ± 2 seconds to 26 ± 3 seconds. Previously (Exp. Med. and Surg. 17:219, 1959) normal guinea pigs were shown to have less prothrombin complex activity than most mammals, and prothrombin time could not be reduced by Vitamin K. However, prothrombin times determined with Russell venom thromboplastin yielded 15 ± 2 seconds in both untreated and 4-aminoantipyrine treated guinea pigs. This suggests that the normal guinea pig has less proconvertin activity than other species and that this activity is increased by the drug.

4 **Physiology**

EFFECTS OF INTRAVENOUS INJECTIONS OF THE ANTICOAGULANT FRACTION OF INCUBATED FIBRINOGEN (AFIF). D.C.Triantaphyllopoulos (introduced by J. W. Pearce.) Dept. of Physiology and Pharmacology, University of Alberta, Edmonton, Canada.

The fraction of incubated fibrinogen precipitated between 25 and 50% ammonium sulfate saturation (AFIF) inhibits the coagulation of blood in vitro (Triantaphyllopoulos, D.C., Can. J. Biochem. & Physiol., 36:250, 1958). One half to 0.65 gram per Kg. body weight of AFIF prepared from Armour's bovine fibrinogen was injected intravenously into rabbits. The anticoagulant effect was apparent immediately and lasted in the animal for 2 1/2 to 5 1/2 hours. Blood collected in glass tubes remained unclotted for at least 24 hours. Surgical wounds inflicted prior to the injection did not bleed. Autopsies also failed to reveal any internal hemorrhage. The bleeding time, however, was prolonged when tested by cutting the marginal vein of the ear. Coagulation studies showed: (1) clottable fibrinogen normal; (2) thrombin clotting time infinite; (3) one stage prothrombin time over 1 minute; (4) failure of protamine (0.25 mg./ml.) to correct the thrombin clotting time; (5) the plasma precursors of plasma thromboplastin normal in oxalated but very low in native plasma; (6) the serum precurosors normal; (7) the platelets in blood smears normal in number and appearance.

5 **Physiology**

Impurities in Heparin. Helen J. Bell (intr. by L. B. Jaques). Dept. of Physiology, Univ. of Saskatchewan, Saskatoon, Sask., Canada.

Chromatography of the heparins of several species on paper by the method of Bell (L. B. Jaques and H. J. Bell Methods of Biochemical Analysis 7, 253, 1959) showed relatively large spots with mucicarmine (neutral and acidic polysaccharides), 8-hydroxy-quinoline and kojic acid (inorganic materials), alizarin-ammonia (calcium and sodium salts of weak acids) and ammoniacal silver nitrate-fluorescein (chloride). Elution experiments proved these areas were not associated with anticoagulant or metachromatic activity and hence were regarded as impurities in the heparin. The species studied included dog, beef, pork, sheep, heparin monosulfuric acid, beef β-heparin and sheep β-heparin. The results obtained led to the conclusion that comparison of the species can only be made on purified samples. Fractions removed from commercial beef heparin were also studied chromatographically.

6 **Physiology**

QUALITATIVE DIFFERENCES BETWEEN THE ACTION OF VARIOUS PLASMINOGEN ACTIVATORS ON HUMAN PLASMA AND ON FIBRIN CLOTS. W. D. Sawyer*, N. Alkjaersig*, A. P. Fletcher* and S. Sherry. Washington Univ. School of Med., St. Louis, Mo.

Thrombi formed from plasma contain plasminogen, as does the surrounding plasma. Plasminogen activators added to a system containing plasminogen in these two phases (thrombus and surrounding plasma) may activate the plasminogen in either phase preferentially or equally. Preferential action of plasminogen activator in one or other of the phases causes different physiological effects: activation of clot plasminogen causes thrombolysis (clot dissolution), whereas activation of plasma plasminogen produces general proteolytic effects. Three plasminogen activators, naturally occurring plasminogen activator, urokinase and streptokinase, have been studied in vitro (and where possible in patients) with regard to their ability to effect the activation of plasminogen in a dual phase system (fibrin clots in plasma). Isotopically labelled reagents were used to facilitate the differentiation of effects. The results indicate that, in low concentrations, plasma activator and urokinase will activate plasminogen in a thrombus without activation of plasma plasminogen, while the converse may be observed with streptokinase. At higher concentrations, all three activators will activate the plasminogen of both plasma and thrombus.

7 **Physiology**

FACTORS INFLUENCING POLYMER AND GEL FORMATION FROM FIBRIN MONOMER. Z.S.Latallo, N.Alkjaersig and A.P.Fletcher (intr. by S.Reichlin) Washington Univ. School of Med., St. Louis, Mo.

Recent studies (J. Clin. Invest. 38: 1005, 1959) indicate that the products of fibrinogen proteolysis interfere with polymer/gel formation from fibrin monomer. This coagulation anomaly accounts for most of the features of the hemorrhagic diathesis observed in "fibrinolytic disorders". Polymer/gel formation rates are difficult to study as isolated events. Consequently, an assay procedure has been devised which measures the effects of various agents on the rate of fibrin polymerization of a standardized fibrin monomer solution. Fibrin monomer was prepared by dissolving clots made from purified fibrinogen in 1M sodium bromide at pH 5.3. Polymerization rates were studied in a spectrophotometer at 3500 A^{o} and 6000 A^{o} after adjustment of the solution to pH 6 and 0.1M (serial increases in O.D. results from the altered light scattering properties of the polymerizing solution). The addition of interfering substances (fibrinogen proteolysis products, urea, etc.)or enhancing substances (dextran) to the test solution causes deviations from the control slopes. Quantitation of this divergence assays the effect and serves to distinguish between substances interfering with thrombin action and those interfering with fibrin polymerization.

8 **Physiology**

FAILURE OF HEMOSTASIS IN THE URINARY TRACT: THE ROLE OF UROKINASE. G. P. McNicol, A. P. Fletcher and S. Sherry (intr. by C. A. Moyer) Washington Univ. School of Med., St. Louis, Mo.

Persistent and severe hematuria may follow surgical and other urinary tract trauma, but its pathogenesis is obscure. Urine, due to its content of urokinase (a plasminogen activator), has the capacity to induce the lysis of fibrin clots. Theoretically, clots formed in a traumatized area of the urinary tract could be lysed by urokinase action and hemostasis thereby impaired. Epsilon-aminocaproic acid (EACA), an inhibitor of plasminogen activation, has been used to study the role of urokinase in the pathogenesis of this hemorrhagic diathesis. Estimation of urokinase excretion rates in patients subjected to transurethral prostatic resection showed no alteration from normal, but in similar patients treated with EACA, either locally or intravenously, complete inhibition of urokinase activity occurred (fibrin plate test). Dialysis of the urine to remove EACA revealed that urokinase excretion had not been impaired by the EACA administration. Since the EACA treated patients experienced a substantial reduction of urinary hemoglobin loss over control patients, the findings indicate that urokinase action may contribute significantly to impaired hemostatic mechanisms in the urinary tract.

XUM

9 **Physiology**

THE PURIFICATION OF HUMAN PLASMINOGEN AND BOVINE PLASMINOGEN. N. Alkjaersig (intr. by J. R. Smith) Washington Univ. School of Med., St. Louis, Mo.

Current methods used for the purification of human plasminogen result in preparations of high specific activity, which are sparingly soluble and relatively unstable at neutral pH. Since these methods employ an acid extraction of plasma Fraction III (Cohn), the possibility exists that the physicochemical properties described for human plasminogen might be dependent on alterations induced by the purification procedure. Previous methods used for the purification of bovine plasminogen have been relatively unsatisfactory and physico-chemical characterization of the proenzyme has not been accomplished. Serum euglobulin fractions from human and bovine plasma have been chromatographed on substituted cellulose ion exchangers (best results with DEAe). Gradients of diminishing pH 8.5-7.0 and increasing ionic strength 0.01M-0.3M were used. Human plasminogen with a specific activity 150-180 casein units/mg tyrosine (equivalent or superior to that achieved by acid fractionation) and bovine plasminogen with a specific activity of 120 casein units/mg tyrosine have been prepared. Preliminary characterization studies indicate that the electrophoretic mobilities (over a limited pH range) are similar for human plasminogen prepared by either the acid extraction procedure or chromatography; the two preparations differ, however, with regard to stability and solubility at neutral pH.

10 **Physiology**

SOME CHANGES IN COAGULANT AND FIBRINOLYTIC POTENTIALS FOLLOWING SUDDEN DEATH. J. M. McKenzie, M. M. Guest, and D. R. Celander (intr. by E. L. Porter.) School of Aviation Medicine, Brooks Air Force Base, Texas, and Univ. of Texas Med. Branch, Galveston.

Apparently healthy dogs were kept in reasonably quiet surroundings on an adequate diet for 45 days or longer. When these animals were killed by sudden exposure to a simulated high altitude, the fibrinogen content of the stagnant venous pool fell progressively, the average half-time of fibrinogen disappearance being 63 minutes after death. Few coagula were noted at necropsy. In contrast, dogs which had been exposed to chronic, intermittent electric shock exhibited no post-mortem fall in fibrinogen until about 47 minutes after death when a massive coagulum formed through the heart and major vessels. Disappearance of fibrinogen in the cadavers of unstressed dogs was impeded by selected doses of heparin administered before death. Ante-mortem injections of purified bovine antifibrinolysin retarded the disappearance of fibrinogen and promoted the formation of coagula after death. These results indicate that both coagulant and fibrinolytic activity are involved in the loss of blood coagulability in the cadaver. (Supported by Contract AF 41(657)-228 between the United States Air Force and the University of Texas Medical Branch).

11 **Physiology**

DEMONSTRATION OF PROTHROMBIN IN LIVER PARENCHYMAL CELLS USING FLUORESCENT ANTIBODY. Marion I. Barnhart and Gordon F. Anderson.* Dept. Physiology and Pharmacology, Wayne State University, Detroit, Michigan.

Previous studies while implicating the liver have not identified the cell type responsible for synthesis of any one of the trace plasma proteins concerned in blood coagulation. In this study to find out the cellular site for prothrombin synthesis fluorescent antibody and other immunochemical procedures were utilized. With dog prothrombin as the antigen high-titered antiprothrombin sera were produced in rabbits. Dog prothrombin mixed with $Al(OH)_3$ and injected IM gave potent antisera. Globulin concentrates were coupled with rhodamine (R*) and applied to dog liver sections or imprints. Such R*-antiprothrombins precipitated out on parenchymal cells containing sufficient prothrombin. Adsorption of R*-antiprothrombin by dog prothrombin reduced the specific orange fluorescence. Uncoupled antiprothrombin serum suppressed fluorescence. Autofluorescence varied with the fixatives. It appears that R*-antiprothrombin is specific in its reaction with prothrombin of the liver parenchymal cell. However, liver parenchymal cells did not react uniformly with R*-antibody. This may mean that a certain type of liver parenchymal cell produces prothrombin, or that there is cyclic production of prothrombin. (Supported by USPHS Grant H3447 NIH)

12 **Physiology**

PURIFICATION OF ASPERGILLIN O, A NEW MOLD FIBRINOLYTIC AGENT. D.Adamis*, M.Stefanini, J.F.Horace*, H.M.Marin*. Jos.Stanton Mem.Lab. St.Elizabeth's Hospital and Tufts University School of Medicine, Boston.

One strain of Aspergillus oryzae(B-1273) excels as a producer of fibrinolytic material. We have obtained a purified preparation of the active agent. The fungus is grown on a liquid medium (Horace, J. and Stefanini, M., Proc.Soc.Exp. Biol.& Med. 102, 201, 1959) in the dark for 5 days at which time culture is separated by filtration through Whatman paper No.40. The pH of the yellow medium is brought from 5.4 to 6.8 by adding 1/10 volume of normal/10 NaOH, slowly and with constant stirring. When 2 volumes of acetone are added, also slowly and with constant stirring, a white flocculate is obtained. After settling overnight at 4°C, suspension is centrifuged at 1,000 r.p.m./10 min. and precipitate is dried for 1 hr. under aspiration and resuspended in a volume of distilled water equal to 1/10 of original filtrate. 25 mgs. of carbon,finest grade, are added per ml. After separation of carbon, liquid is dialyzed for 12 hrs. and lyophilized. Final material is whitish, fluffy and hygroscopic and average yield is 30-60 mgs/liter of filtrate.

Physical properties of Aspergillin O have been established and much information has been obtained on chemical properties and composition. These data will be reported in detail.(aided by grant from the American Heart Association).

13 **Physiology**

FIBRINOLYTIC EFFECT OF PLASMIN-ANTIPLASMIN COMPLEX. Clara M. Ambrus, N. Back, and J. L. Ambrus. Roswell Park Memorial Institute and University of Buffalo Grad. and Med. Schools, Buffalo, New York.

Preparations of human plasmin were mixed with antiplasmin in proportions which neutralize the in vitro caseinolytic activity. In dogs carrying experimental clots formed with I^{131} labeled fibrin, these preparations caused dissolution of clots as evidenced by disappearance of radioactivity and reestablishment of blood flow. There was no change in circulating fibrinogen, prothrombin or other clotting factor levels. When plasmin alone was injected, lysis of clots was accompanied by some decrease of the level of several of these factors. I^{131} labeled plasmin-antiplasmin complex infused into dogs with non-radioactive fibrin clots resulted in localization of I^{131} on the clots. It appears that fibrin removes competitively plasmin from its complex form with antiplasmin. This mechanism protects circulating clotting factors from proteolysis by plasmin while the latter is available for fibrinolysis. (Supported by grants from the Am. Hearts Assoc., Natl. Inst. of Health, U.S.P.H.S., Parke Davis and Co. and Merck, Sharp and Dohme Co.)

14 **Physiology**

ISOLATION OF PURIFIED PLASMIN. Daniel L. Kline and Jacob B. Fishman*, Yale University School of Medicine, New Haven, Conn.

When streptokinase (SK) is added to human plasminogen, the resulting mixture contains a plasminogen activator as well as plasmin. We used minimal amounts of SK so that only enough activator was formed to convert the plasminogen present to plasmin. 25 SK units/mg. enzyme (3 SK units/ casein unit) activated about 80% of the plasminogen. The remainder was converted to plasmin during overnight dialysis of the activated suspension vs. H_2O. The pH was adjusted to 9.5 and plasmin precipitated from this clear solution by the addition of NaCl solution to a final salt concentration of 0.021M. The average plasminogen purity at the start was 53 casein units/mg.N; plasmin isolated, 145 units/mg.N; yield, 64%. No activator activity has been detectable in the plasmin preparations. Purified plasmin is sparingly soluble in H_2O. Clear solutions are obtained below pH 4 or above 9. Stability studies are under way. (Supported by Grant NSF-G7496 to D.L.K.).

15 *Biochemistry*

CHROMATOGRAPHY OF PARTIALLY PURIFIED BOVINE PLASMINOGEN (PROFIBRINOLYSIN). E. R. Cole*, A. Dalby*, R. W. Fuller* and E. T. Mertz. Department of Biochemistry, Purdue University, Lafayette, Indiana.

A 30% ammonium sulfate precipitate from bovine serum has been subjected to isoelectric precipitation at pH 5.3 at a 40-fold dilution, resulting in a 3-fold purification. This isoelectric precipitate was chromatographed on small columns of calcium phosphate gel (hydroxylapatite form). After a preliminary elution of inactive protein with 0.05 M sodium phosphate buffer, pH 6.8, the plasminogen was eluted with 0.1 M buffer. The recovery of plasminogen from the isoelectric precipitate was 48% and the specific activity of the solution was 12 esterase units/unit optical density, as measured by a manometric method (Dalby and Mertz, Am. J. Physiol. 191 505 (1957)). The isoelectric precipitate has also been subjected to chromatography on DEAE-cellulose columns. On such columns, plasminogen can be eluted in the range of 0.03-0.05 M sodium phosphate buffer, pH 7.4. The recovery of plasminogen, after dialysis and lyophilization, averaged 40% and the specific activity averaged 5-6 esterase units/unit optical density. Elution ranges narrower than 0.03-0.05 M increased the purity of the eluted plasminogen but decreased yield. (Supported by Contract DA-49-007-MD-195, Office of the Surgeon General, Department of the Army).

16 *Biochemistry*

FIBRINOLYSIS AND ARGININE RELEASE: INHIBITION STUDIES
S. I. Cohen and R. Warren (introduced by R. M. Ferry)
Harvard Medical School & W. Roxbury VA Hospital, Boston, Mass.

Recalcification and coagulation of human blood plasmas containing the plasminogen activator streptokinase results in plasma clots that disintegrate and dissolve after formation. More unbound arginine and lysine are released in such plasmas than in plasmas recalcified in the absence of streptokinase where the clots that form do not disintegrate and dissolve (Fed. Proc. 18, 205, 1959). Attention was focused on inhibiting the plasmin system in human blood plasma as a step toward elucidating the underlying enzymatic mechanism(s) responsible for the amino acids liberated as the result of coagulation and/or plasma clot lysis. The results are: 1. Coagulation of plasmas containing purified streptokinase and added amounts of E-amino caproic acid, lysine, ornithine, or soybean trypsin inhibitor results in clots that form and do not dissolve and the concentrations of unbound arginine in the resulting sera remain the same as in the initial plasmas. 2. Glutamine, glutamic acid, and histidine demonstrate no inhibitory effect on plasma clot lysis and arginine release. It is concluded that the arginine and lysine released after recalcification and coagulation of human blood plasma is attributable to the plasmin system which is somehow triggered into limited action by the coagulation process.

17 *Biochemistry*

PHOSPHOLIPIDS IN BLOOD COAGULATION. Karl H. Slotta. Univ. of Miami School of Med., Coral Gables, Fla.

Phosphatidyl choline (PC), phosphatidyl serine (PS), and phosphatidyl ethanolamine (PE) isolated independently are inactive in the Thromboplastin Generation Test. Small amounts of PS in combination with PC and/or PE are necessary for a fast generation of thromboplastin. Fully active thromboplastin preparations were obtained after one minute incubation time with suspensions of 10% PS in PE or in PC. All naturally occurring mixtures of phospholipids (PLs) from animal and vegetable sources are rich in PC and PE. The difference in their thromboplastic activity depends thus only on how much PS they contain. Brain and platelets are comparatively the richest sources of PS and they have also the highest thromboplastic activity. The PLs from fresh eggs are scarcely active, and the presence of no PS could be ascertained; however, the activity increases with the development of the chicken embryo. The total PLs and the cephalin part of soybeans, with about 0.75% and 2% PS respectively, are active. Although the amount of PE in the lecithin and cephalin part of soybean PLs is almost the same, the lecithin part is completely inactive, evidently because it is free of PS. (Supported by NIH Grant H-3052.)

18 *Biochemistry*

MOLECULAR CHANGES IN ACTIVATION OF PLASMINOGEN. Ubaldo Rifé* and Sidney Shulman. Department of Biophysics, University of Buffalo School of Medicine, Buffalo, N. Y.

Activation of human plasminogen by streptokinase involves the removal of approximately 25% of the molecule, as has been shown either by an accumulation of TCA-soluble material to this extent or by the decreased molecular weight of plasmin. In the present studies activated samples were adjusted to 1 N in HCl and 1 M in NaCl to precipitate the plasmin. The supernatant solution was dialyzed free of salt, re-concentrated, and then examined by various procedures. The dialysis membrane retained 60% of the supernatant material. In the ultracentrifuge, a single peak was seen, with a sedimentation coefficient of about 1.5 S. Optical electrophoresis at pH 2 revealed three components, all having mobilities lower than those of plasminogen or plasmin. Chromatography on Dowex columns has given separation into three main peaks of UV-absorbing material. It is concluded that a large portion of the non-plasmin product consists of a group of large-size peptides, at least three in number. (Supported in part by a Research Grant H-1792 from the National Heart Institute, P.H.S.)

19 *Biochemistry*

HUMAN PLASMINOGEN - PHYSICOCHEMICAL STUDIES. W. Baumgarten, R. B. Cole, A. Hogans, A. Danforth and D. S. Spicer (intr. by C. C. Porter.) Merck Institute for Therapeutic Research, West Point, Pa.

Human Plasminogen may be prepared by various methods. Considerable purification (Vox Sanguinis, vol. 4, no. 2, 1959) is achieved over the starting material, however, our purest preparations are still nonhomogenous. Electrophoretic analysis in veronal buffer (pH 8.6) indicates as many as six components, whereas at pH 2.0 in glycine buffer the same preparation yields relatively simple patterns. Considerable additional purification may be achieved by fractional ultracentrifugation. The component(s) of Cohn's Plasma Fraction III-3 associated with fibrinolytic activity has been indicated by ultracentrifugation, electrophoresis and ion exchange chromatography. The data obtained by the above methods will be presented and discussed.

20 *Biochemistry*

Anticoagulant and Anticephalin Activity of Normal Human Plasma Fractions Separated by Ultracentrifugation. L.A. Kazal, O. P. Miller, *R.H. Holburn* and L.M. Tocantins* (with technical assistance, R. Curry, A. Pfiefer), Cardeza Foundation, Jefferson Medical College, Philadelphia, Pennsylvania

Citrated normal human plasma was subjected to ultracentrifugation (Spinco, Model L, #40 rotor, 144,000 x g, 23 hr.) separated into 4 or 5 layers, analyzed and assayed (Fed.Proc. 18,1018(1959). Upper fractions #1 and #2, and frequently #3, exhibited anticoagulant and anticephalin activities; lower fractions usually accelerated clotting. The two uppermost fractions are primarily chylomicron and alpha-2 lipoproteins. Fraction 3 consists of both alpha-1 and beta lipoprotein as well as other plasma proteins. Lipoprotein fractions were also separated by ultracentrifugation of plasma at NaBr densities; 1.006, 1.018, 1.063, and 1.21. After dialysis against 0.022 M sodium citrate-0.15 M NaCl, only the alpha-1 and alpha-2 fractions consistently showed anticephalin activity. Alpha-2 inhibited thromboplastin generation; alpha-1 did not. Recalcified mixtures of these lipoproteins and normal plasma were not consistently anticoagulant. (Supported by N.I.H Grant H-3544)

XUM

21 *Biochemistry*

MECHANISM OF BOVINE AND HUMAN PLASMINOGEN ACTIVATION BY HUMAN PLASMIN AND STREPTOKINASE. W. F. Blatt,* J. Zylber,* J. L. Gray* and H. Jensen. US Army Med. Rsch. Lab., Fort Knox, Ky.

Equilibration of plasmin-streptokinase mixtures at pH 2.0 led to a loss in the ability to convert bovine plasminogen to plasmin. The activity could be re-established after adjustment to pH 6.6 upon readdition of streptokinase. Results of repeated low pH treatments and readditions of streptokinase do not support the hypothesis of a specific proactivator conversion. These studies were extended to human plasminogen, present in a purified fibrinogen preparation. Lysis times were determined in the thrombelastograph. The results supported the conclusion that there is prior interaction of plasmin with streptokinase in the conversion mechanism. Application of thrombelastography to isolated clotting and lytic systems would appear to provide a sensitive procedure for measuring activities of various factors of the blood coagulation system.

22 *Biochemistry*

KINETIC BEHAVIOR OF THE SYSTEM:FIBRINOGEN, THROMBIN, FIBRINOLYSIN. Douglas M. Surgenor. Harvard Medical School and Protein Foundation, Boston, Mass.

In the fibrinolytic system as usually operated it is assumed that fibrinogen(F) is first converted to Fibrin(Fn) by Thrombin(T); then Fn is split by fibrinolysin(E) to products (P). Actually, all species between F and Fn are under attack by E from zero time. Measurement of E by lysis time of clot is theoretically unsound. On the other hand the best index of activity, the initial rate of splitting of Fn, is not usable since $dFn/dt = k_1TF - k_2EFn$. In a search for an accurate parameter by which to measure E, the behavior of a simple model: A $\xrightarrow{k_1}$ B $\xrightarrow{k_2}$ C has been studied in an analog computor. As expected, the maximum concentration of B is obtained when k_1 is large in comparison to k_2. When $k_1/k_2 = 40$, the concentration of B reaches 0.9A before dB/dt becomes negative. When $k_1/k_2 = 10$ the peak concentration of B is 0.72A. Analysis of the computor curves revealed a linear relation between k_2 and -dB/dt at B = 0.5A, provided the maximum concentration of B had been 0.65 or higher. Good correlation was also seen between k_2 and t for B = 0.5A. In the real system, optical density has been used to follow Fn vs. time, with results in good agreement with predictions from the model. This provides a more quantitative approach to the kinetics of fibrinolysis. (Supported by USPHS grant H-2440.)

23 *Biochemistry*

PROFIBRINOLYSIN-FREE FIBRINOGEN. D.R. Celander and M.M. Guest (intr. by D. B. Calvin). Univ. of Texas Med. Branch, Galveston

Fibrinogen and fibrin are often preferable substrates in studying fibrinolysin. Because readily available preparations of fibrinogen and thrombin contain profibrinolysin, the proteolysis of fibrinogen or dissolution of fibrin clots by systems of ill-defined composition may be attributed either to the action of kinase or to that of a proteolytic enzyme present in the material or both. Hence simple methods of removal of profibrinolysin from fibrinogen and thrombin are of value. With the aid of chromatographically purified commercial thrombin and Seegers' thrombin (which contains no detectable profibrinolysin), purified urokinase and streptokinase-treated, highly diluted human plasma fractions, systems sensitive to the presence of profibrinolysin have been constructed in which the response of fibrinogen can be studied during experiments designed to free it of profibrinolysin. Purified human fibrinogen obtained by multiple adsorption of the freeze-thaw product with $CaPO_4$ gel has no detectable profibrinolysin and less than 1/600 of the prokinase activity present in the starting fibrinogen. Similar treatment of freeze-thaw and Armour's bovine fibrinogen gave preparations in 50% yields which were not lysed in 7 days by potent preparations of urokinase and plasma kinase. Concentrated urokinase solutions readily bring about dissolution of profibrinolysin-free clots in 2-5 hours.

24 *Biochemistry*

EFFECT OF ELECTROLYTES ON THE CLOTTING ACTIVITY OF RUSSELL'S VIPER VENOM ("STYPVEN"). Gottfried Härtel (intr. by Ancel Keys.) Lab. Physiol. Hygiene, U. of Minn., Minnesota.

To 0.2 ml. of citrated plasma were added 0.2 ml. of $CaCl_2$ (optimal concentration) and 0.2 ml. of Stypven solution, with varying concentrations of Stypven and NaCl, and clotting time was measured. For each Stypven concentration the minimum clotting time (highest activity) was found at NaCl concentration between 0.1 and 0.5%. When Stypven was depleted of salt by dialysis any addition of NaCl, KCl, NaBr, Na_2SO_4, or $MgSO_4$ prolonged the clotting time and equal ionic strengths of these salts gave equal clotting times. Commercially available Stypven contains 50 to 60 times more NaCl than the stated amount of active substance. The occasionally reported inhibitory effect of high Stypven concentration in clotting tests is apparently explained by the salt content of the product.

25 *Pharmacology*

ORAL HEPARIN AND THE LIPEMIA CLEARING FACTOR. Thomas Toft*, James Shane* and A.R.McIntyre. Univ. of Nebraska Coll. of Medicine, Omaha Nebraska.

Evidence was obtained on normal volunteers that the oral administration of enteric coated heparin tablets resulted in a diminution of the lipemia resulting from the ingestion of cream. For each of 2 days prior to the ingestion of approximately 240 ml. of standard cream, (18% fat) four 5000 unit enteric coated heparin tablets were taken (1 before each meal and 1 at bed time). Samples of blood were obtained by venepuncture and the clotting time (Lee-White) and optical density (at 650 millimicrons) were determined. In twelve normal subjects the average clotting time was 14.1 mins. In the same group, following oral heparin, 13.6 mins. After the ingestion of cream the clotting time was 10.4 mins. and 11.4 mins. before and after heparin respectively. The probability of these differences having significance is small. However the optical density following the ingestion of cream rose from a normal value of 0.062 units to 0.49 units. When the cream was taken after heparin-ingestion, in the above doses, the average optical density was only 0.32 units. This difference is highly significant (P=0.001). It is concluded that the lipemia evoked by the ingestion of cream and determined by optical density is decreased by the prior administration of oral heparin.

26 *Pathology*

THROMBOPLASTIN FORMATION WITH RUSSELL'S VIPER VENOM. Andrev C. Peacock (intr. by G. Z. Williams.) Natl. Cancer Inst., Bethesda, Md.

An incubation mixture of aluminum hydroxide adsorbed plasma, serum, phospholipid, Russell's viper venom and calcium rapidly produces prothrombin converting activity. The concentration of the several reactants was varied, and the resulting changes in the rate of development and the amount of thromboplastic activity measured. The reaction did not proceed in the absence of any reactant. The serum and the adsorbed plasma affected both yield and rate, but the serum factor seemed more effective in controlling the rate. The concentration of phospholipid affected only the yield. The effect of the Russell's viper venom appeared to be enzymatic. The reaction could be divided into two phases; the first involved all factors except phospholipid, and the second, the reaction between the phospholipid and the product of the first reaction. The serum factor is probably Stuart-Prower factor, the plasma factor is probably Factor V.

27 *Pathology*

SERUM THROMBOPLASTOGENIC ACTIVITY. H. C. Pirkle, V. G. Allen, H. C. Anderson and J. J. McHugh (intr. by W. M. Christopherson.) Dept. of Pathology, Univ. of Louisville, Louisville, Ky.

The following experiments were designed to distinguish the factor(s) responsible for thromboplastogenic activity of fresh serum from the thromboplastin generating factors in the system of Biggs and Douglas. Incubation mixtures of 1-hour-old human serum, $CaCl_2$, and washed platelets were assayed for thromboplastic activity by determining the time required for aliquots to promote clotting of samples of recalcified Dowex-50 plasma. Potent thromboplastic activity develops in such mixtures. Treatment of serum with $Al(OH)_3$ abolishes its thromboplastogenic activity. Activity is not restored to treated fresh serum by adding aged serum or $Al(OH)_3$-treated plasma, although both are fully active in the thromboplastin generating system of Biggs and Douglas. Thus, the thromboplastogenic activity of fresh serum cannot be ascribed to any of the individual factors involved in the thromboplastin generation test or to either of the groups of factors present in aged serum or adsorbed plasma. (Supported by grants from the National Heart Institute and the Kentucky Heart Association.)

28 *Pathology*

SPONTANEOUS BLEEDING DURING ADMINISTRATION OF HEPARIN. H. Yoshimura and I. Djerassi (intr. by Sidney Farber.) Children's Cancer Res. Fndn. and Dept. Pathology, Harvard Medical School, Boston, Mass.

Spontaneous bleeding in patients in the course of prolonged and intensive anticoagulant therapy suggests increased vascular fragility in addition to, or associated with, defective coagulation. In this study, changes in composition of the lymph in animals were used for evaluation of vascular integrity. The effects of repeated administration of high doses of heparin on content of red blood cells in lymph was investigated in rats. Lymph was collected by cannulation of the main abdominal lymph duct. Heparin was given intravenously at regular intervals. Doses larger than 0.6 mg/kg, given every 30 minutes, usually brought about a gradual change of lymph from clear-white to frankly bloody within 3 to 8 hrs. The bleeding in the lymph persisted for more than 24 hrs. despite return of the coagulation mechanism to normal. In a number of experiments a decrease of circulating platelet levels from an average of 800,000 to 500,000 was observed. Bleeding occurred also without simultaneous changes in platelet level.

Further studies on the relationship of blood coagulation and vascular integrity are in progress.

29 *Pathology*

PROTHROMBIN HOMEOSTASIS IN CROSS-CIRCULATED NORMAL AND DICOUMAROL TREATED DOGS. R.D. Langdell, L.W. Bowersox*, R.A. Weaver*, and R.E. Price*. Dept. of Pathology, Univ. of North Carolina, Chapel Hill, N.C.

In order to obtain additional information on the rate of synthesis and in-vivo utilization of prothrombin, we have used the technique of cross-circulation. Arterio-venous shunts of polyethylene tubing were made between normal dogs and dogs made prothrombin-deficient by administering dicoumarol. The shunts were allowed to function for various time intervals to allow mixing of the circulating blood. Prothrombin was determined by the 2-stage method on samples obtained at various time intervals after the cross-circulation had been stopped. The prothrombin of the dicoumarol treated animal was elevated to about 50 per cent levels by the cross circulation but fell to the initial deficient level in 6 to 12 hours. It required about 72 hours for the non-dicoumarol treated partner to return to its pre-operative prothrombin level. The relationship of these findings to the homeostasis of prothrombin will be discussed. (Aided in part by Grant H-1648, NIH, PHS.)

30 *Pathology*

BIOLOGICAL PROPERTIES OF ASPERGILLIN O, A NEW MOLD FIBRINOLYTIC AGENT. F. Soardi*, M. Stefanini, D. Adamis* Jos.Stanton Mem.Lab. St.Eliz.Hosp. and Tufts Univ.Sch.Med. Boston.

Aspergillin O, a material with fibrinolytic activity from Aspergillus oryzae(B-1273) has been recently purified. Purified Aspergillin O digests bovine and human fibrin heated plates at a dose as low as 0.097 μg. Against unheated plates it acts more slowly and more strongly. Its effect is directly lytic as the substance is not an activator of the fibrinolytic system. Its activity against human and dog plasma clots is limited by inhibitors but greatly enhanced by small quantities of heparin. In final concentration of 100 μg, Aspergillin O digests casein, egg albumin, denatured human hemoglobin, TAME, BAME, is inactive against normal human hemoglobin. Activity is unaffected by soya bean inhibitor, δ-amino n-valeric acid and protamine sulfate, only slightly by ε-amino n-caproic acid. Material accelerates slightly the generation of thromboplastin and thrombin but, on incubation with plasma, shows strong antithrombin activity. The following coagulation factors are destroyed or inhibited: fibrin, fibrinogen, labile factor, prothrombin, stable factor. AHG, PTC, Hageman and Stuart factors are relatively resistant. At concentration of 1 mg/ml Aspergillin O demonstrates lipolytic activity in plasma. (Aided in part by grant from the American Heart Association).

31 *Nutrition*

MECHANISM OF CLOT-ACCELERATING EFFECT OF BUBBLING. Paul Didisheim (intr. by G. E. Cartwright.) Dept. of Medicine, University of Utah, Salt Lake City, Utah.

Blood circulating in vivo clots slowly if at all; shed blood clots quickly after collection. Two factors involved in initiating coagulation ex vivo are the blood-solid and blood-gas interfaces. All glassware used in this study was siliconized to minimize the clot-accelerating effect (CAE) of glass and to study only the CAE of varying gases. Bubbling O_2, CO_2, N_2 or compressed air through normal whole blood shortened the coagulation time from >20 min. to <6 min. Low-spun (190xG) platelet-rich citrated plasma clotted in 22 min. after adding $CaCl_2$; if air was continually bubbled through the plasma following addition of $CaCl_2$, the clotting time averaged 7 min. If air was first bubbled through the plasma for 5 min. and $CaCl_2$ then added, the clotting time averaged 12 min. A steady fall in platelets occurred during bubbling. In contrast, there was no CAE of bubbling either prior to or following addition of $CaCl_2$ if bubbled plasma was higher-spun, platelet-poor (1700xG). Thus the mechanism of the CAE of bubbling appears distinct from that of contact of blood with glass: the latter is known to be dependent upon a plasma factor and is demonstrable in the absence of platelets, whereas the former is demonstrable only in the presence of an easily sedimentable substance, presumably platelets.

32 *Immunology*

THE EFFECT OF LOW TEMPERATURE ON THE INTRINSIC THROMBOPLASTIN SYSTEM. Lamont W. Gaston and Helen DeOrsay (intr. by S. Leskowitz.) Massachusetts General Hospital and Harvard Medical School, Boston, Massachusetts.

It is known that increased activity of Hageman factor and plasma thromboplastin antecedent (PTA) secondary to contact activation by glass or silicon dioxide (SiO_2) will cause an apparent increase in antihemophilic factor (AHF) and plasma thromboplastin component (PTC) when the latter two factors are assayed by methods utilizing an incomplete thromboplastin. It was observed in this laboratory that silicone plasma stored for a week at -68° will, in contrast to silicone plasma stored at -20°, demonstrate an apparent 100 per cent increase of AHF activity and a moderate to negligible increase of PTC. In studying this phenomenon, it was observed that such activity can be removed by adsorption onto SiO_2 and $Al(OH)_3$. AHF-like activity is found in serum stored at -68° which is known not to contain true AHF. AHF activity could not be demonstrated in hemophilic A plasma stored at -68°. Preliminary results indicate that the apparent increase in AHF activity is a reflection of Hageman factor and PTA activation. It is concluded that such activation accounts for the apparent increase of AHF and PTC in plasma stored at -68°. (Supported by a grant from the U. S. Public Health Service.)

33 *Immunology*

COLUMN CHROMATOGRAPHY OF PLASMINOGEN AND PLASMIN. Philip S. Norman (intr. by Leighton E. Cluff) Dept. of Medicine, Johns Hopkins Univ., Baltimore, Maryland.

Plasminogen, partially purified by the method of Kline (J. Biol. Chem., 204:949, 1953) was subjected to chromatography on diethylaminoethyl cellulose. The protein adsorbed readily to the cellulose at pH 7.0 but was not eluted by NaCl solutions or phosphate buffers. 3-amino propanol is an inhibitor of plasmin activity and also increases the solubility of plasminogen in neutral salt solutions. Stepwise elution with 0.5 M 3-amino propanol at pH 7.0, 0.5 M 3-amino propanol at pH 2.0, and 0.01 M HCl produced a chromatogram with 3 symmetrical protein peaks, A, B, and C. The 3-amino propanol was removed by dialysis against 0.001 M HCl and the 3 components were tested for proteolytic, TAMe esterase, and pro-activator activity after activation by streptokinase. All 3 types of activity were found in component A and components B and C were virtually inactive. These findings are further evidence that esterase and pro-activator activity are not separable from plasminogen itself. Component A was about twice as active proteolytically as the starting material. About 12% of the protein and 30% of the activity were recovered in this component. Plasmin activated by streptokinase was subjected to chromatography by the same method and also produced 3 protein peaks with nearly all of the recovered activity in the first component.

34 *Immunology*

COMPARATIVE ACTIVITY IN VIVO OF THREE FIBRINOLYTIC AGENTS: PLASMIN, TRYPSIN AND ASPERGILLIN O. Marin, H. Stefanini, M. Mueller, L.* Adamis D.* Jos. Stanton Mem. Lab. St. Elizabeth's Hosp. and Tufts Univ. Sch. Medicine, Boston.

Aspergillin O, an agent from Aspergillus oryzae (B-1273) has fibrinolytic activity in vitro. In vivo (dogs) it prevents formation and activity of thrombin and it induces lysis of preformed clots while producing only mild hypofibrinogenemia and deficiency of clotting accelerators. Its activity is enhanced by heparin. Comparative studies were carried out by injecting intravenously in dogs varying doses of plasmin, trypsin and Aspergillin O and following changes induced in coagulation and fibrinolytic mechanisms. At low doses all three agents accelerated conversion of prothrombin to thrombin. At higher doses plasmin and trypsin still accelerated formation of thrombin while Aspergillin O destroyed thrombin formed at rate greater than its production. At very high doses trypsin induced total incoagulability of blood because of massive formation of thrombin and defibrination. Trypsin increased fibrinolytic activity of serum but depressed plasminogen and antiplasmin. Plasmin and Aspergillin O enhanced fibrinolytic and antiplasmin activity without affecting plasminogen level. Thus plasmin and Aspergillin O were comparable in activity but Aspergillin O appeared preferable because of its antithrombin as well as thrombolytic activity.

031. BLOOD–Coagulation–Disorders

1 *Physiology*

OBSERVATIONS ON THE NATURE OF THE THROMBOSIS-INDUCING ACTIVITY OF MAMMALIAN SERUM. Stanley M. Reimer*, Stanford Wessler, and Daniel Deykin*. Beth Israel Hosp., and Harvard Med. School, Boston, Mass.

An in vivo quantal assay of the serum thrombotic accelerator (STA) activity of thrombin-free mammalian sera has been previously described (J. App. Physiol. 14:943, 1959). With this method STA activity is demonstrable in the sera of normal persons; of individuals congenitally deficient in factors V, VII, VIII or X; and of dogs following 4 days of Dicumarol administration. In contrast, STA activity cannot be demonstrated in sera of individuals congenitally deficient in factor IX, Hageman or PTA; of dogs deficient in factors II, VII, IX and X following 6 weeks of Dicumarol administration, or of hens - animals deficient in factor IX, Hageman and PTA. Normal human sera collected on silicone, heated to 56° C for 15 minutes, or adsorbed on $BaSO_4$ also fail to demonstrate STA activity. These results indicate that demonstration of STA requires factor IX, Hageman and PTA, but that STA is not identical with these latter 2 entities. Whether STA is identical with factor IX is not known. Moreover, these findings are in accord with the hypothesis that, in the elaboration of intrinsic thromboplastin, activation of factor IX is dependent on the prior interaction of Hageman and PTA. (Supported by grant #1027 U.S.P.H.S.)

2 *Physiology*

"MILD HEMOPHILIA" AFFECTING BOTH MALES AND FEMALES. Ted P. Bond*, William C. Levin*, D. R. Celander* and M. Mason Guest. Univ. of Texas Med. Branch, Galveston.

Three generations of a family affected with a mild antihemophilic factor (AHF, Factor VIII) deficiency have been studied. In this family a significantly reduced level of AHF was demonstrated in three males and three females. The propositus was a female member of the third generation. All affected members were symptomatic, though the clinical expression of the deficiency was invariably greater in males than females. In each affected individual, abnormalities were found in Lee-White clotting times, prothrombin consumption, partial thromboplastin times (PTT) and thromboplastin generation tests (TGT). These deficiencies were corrected only with those reagents that supplied AHF and were not corrected with plasma from a subject previously diagnosed, in this and other laboratories, as having a severe AHF deficiency. No circulating anticoagulant could be demonstrated. The bleeding times and tourniquet tests were normal. The inheritance pattern does not deviate from that of the sex-linked type. Since it was demonstrated that the males had a more pronounced deficiency than the females, the "sex-linked of intermediate dominance" hypothesis is tenable, although autosomal transmission is not excluded.

3 *Physiology*

A MECHANISM OF PATHOLOGICAL SPONTANEOUS FIBRINOLYSIS IN MAN. Alan J. Johnson, Jack Newman and Daniel P. Schoicket (intr. by L.W. Eichna). Dept. of Int. Med., N.Y.U.-Bellevue Med. Coll. and Dept. of Hemat., Montefiore Hospital, N.Y., N.Y.

Pathological spontaneous fibrinolysis in man, with hypofibrinogenemia and/or gross hemorrhage, may be caused by excessive plasmin in the blood. Hyperplasminemia may, in turn, be due to (1) excessive plasminogen activator, (2) low inhibitor to plasminogen activator and (3) low inhibitor to plasmin. Prolonged pathological fibrinolysis was studied in four patients. Clotted whole blood from each spontaneously lysed in 1/2 hour to 4 hours, and uncontrollable bleeding occurred in two. Activator inhibitor was markedly decreased in all four, while plasmin and activator levels were variably increased. Pathological fibrinolysis was thought to be due primarily to the low levels of activator inhibitor, permitting excessive activation of the fibrinolytic system. To test this hypothesis, a synthetic inhibitor of fibrinolysis (epsilon aminocaproic acid) was infused in each patient in amounts calculated to inhibit primarily plasminogen activation. During and after the infusion, whole blood clot lysis was no longer evident, hemorrhage was promptly controlled, and activator inhibitor and activator levels returned to normal. These data supported the hypothesis that the pathogenetic lesion in these patients was the decreased level of activator inhibitor.

4 *Biochemistry*

THROMBOPLASTINOGENIC DEFECTS OF BLOOD COAGULATION: A NEW TEST FOR THEIR RECOGNITION. Armand J. Quick, Dept. of Biochemistry, Marquette Univ. School of Medicine, Milwaukee, Wis.

When native platelet-poor human plasma is clotted in a silicone-coated test tube, no measurable amounts of prothrombin are consumed and no augmentation results from the addition of platelets or an extract of platelets. When an extract of erythrocytes (hemolysate) is added to the plasma, a high consumption of prothrombin occurs demonstrating that the activity of this agent does not require a contact factor. On this observation a test was developed similar in principle to the basic prothrombin consumption test except that 0.1 ml of a standard preparation of hemolysate is added to 1 ml of native platelet-poor plasma and the clotting carried out in a silicone-coated test tube. With this procedure a low consumption of prothrombin is found in hemophilia, hemophilia B, PTA deficiency, Hageman trait and pseudohemophilia B. The test is not only a sensitive means for detecting deficiencies of thromboplastinogenic factors but it also serves in differentiating this group of defects. In true hemophilia the defective prothrombin consumption is not corrected by adding normal aged serum with the hemolysate, whereas this combination of reagents effects normalization in hemophilia B, PTA deficiency and Hageman trait. (Supported by U.S.P.H. grant H-1612 C13)

032. BLOOD–Coagulation–Platelets

5 *Pharmacology*

EFFECT OF WARFARIN (COUMADIN) SODIUM ADMINISTRATION DURING LACTATION ON BLOOD COAGULATION OF NURSLING RATS. Harold Blumberg, Hyman B. Dayton*, and Samuel M. Gordon*. Endo Labs., Richmond Hill, N.Y.

The use of warfarin (Coumadin) sodium for treatment and prevention of postpartum thromboembolism has raised the question of its effect in lactation. Mother rats were given stock diet with .000125% of added warfarin sodium on the day of littering. After 14 days cardiac blood samples were taken in capillary glass clotting tubes. Clotting time (mean ± S.E.) for 16 control mother rats was 57 ± 4 sec., and 58 ± 2 sec. for 123 control nurslings. Clotting time for 16 warfarin sodium mothers was 185 ± 3 sec., or about 3 times that of the control mothers. Despite prolonged clotting time in the mothers, clotting time in 120 warfarin sodium nurslings was normal, i.e., 57 ± 2 sec. Prothrombin time of warfarin sodium mothers averaged 30%. Similar results on clotting time were obtained after 7 days of lactation in a second experiment. After 14 days of lactation on warfarin sodium diet, some mothers and litters were transferred to stock diet. The young grew normally and in turn produced normal young. When toxic levels of warfarin sodium, e.g., .001%, were fed to lactating mothers, clotting time was prolonged in the nurslings. This demonstrated that the anticoagulant effect was transmitted through the milk when mothers received toxic dosages. However, when a "therapeutic" or "prophylactic" dosage of warfarin sodium was fed to the mothers, there was no effect on the clotting time of the nurslings.

6 *Pathology*

THE THROMBOPLASTIN GENERATION HEPARIN TOLERANCE TEST (TGHTT) IN HYPOCOAGULABLE STATES. J.N. Shanberge and E.E. Regan.* Harvard Medical School, Boston, and VA Hospital, West Roxbury, Massachusetts.

The plasma heparin tolerance test reflects the effect of heparin on all stages of blood coagulation, but has the disadvantage of being influenced by the number of platelets, ergo the amount of platelet factor IV, present in the plasma. The TGHTT is essentially a sensitized thromboplastin generation test (TGT) which amplifies defects in the factors necessary for normal thromboplastic activity production (TG). The influence of platelet factor IV activity is eliminated by substituting cephalin (platelet factor III) for platelets in the generation mixture. Heparin has been shown to affect TG by interferring with the production of PTC (IX) activity. The in vitro effect of a constant amount of heparin (1/40 U. or 0.25 gamma) on TG in normal individuals falls within a definite reproducible range. Patients with chronic liver disease, particularly cirrhosis, display a decreased TGHTT. This may occur even in the presence of normal TG. This latter observation, plus the finding that, in some patients with chronic liver disease, the abnormal TGT may be corrected both in vivo and in vitro with protamine sulfate, is suggested as further evidence for an increased heparin-like antithrombin in such patients. (Supported by PHS #H-2963.)

7 *Pathology*

A LABILE SERUM FACTOR NEEDED FOR THROMBOPLASTIN GENERATION. E. D. Warner, William E. Connor* and John R. Carter. Depts. of Pathology and Internal Medicine, Coll. of Med., State Univ. of Iowa, Iowa City, Iowa.

In the routine performance of the thromboplastin generation test (TGT) using serum aged 3 to 24 hours and soybean phosphatide as a source of platelet factor 3, a serum factor "deficiency" was encountered in many patients. This deficiency was corrected completely by any of the following: aged normal serum, PTC deficient serum, the patient's own serum aged only 1 or 2 hours, and amounts of thrombin (0.024 and 0.048 units) too small to be detected by fibrin clot formation. Prior incubation of the small amounts of thrombin with the serum prevented correction. The TGT was normal when platelets were used instead of soybean phosphatide. Normal $BaSO_4$ absorbed plasma was not corrective. The labile serum defect did not occur in serum aged in a silicone tube. In most of these patients other blood clotting test results were normal. This labile serum defect was found in apparently healthy individuals and in a variety of disease states, e.g. unexplained clinical bleeding, hepatic cirrhosis and polycythemia vera. Care must be taken to differentiate this "labile thrombin" defect from other conditions, especially PTC deficiency.

1 *Physiology*

THE PLATELET DEFECT IN POLYCYTHEMIA VERA. Orhan N. Ulutin * and Shirley A. Johnson. Dept. of Laboratories, Henry Ford Hospital, Detroit, Mich.

An investigation is being carried out on a group of patients who initially presented thrombocytosis in polycythemia vera. In all cases coagulation time, plasma clotting time, prothrombin time and fibrinogen content of plasma and of platelets were in normal range (J. Lab. Clin. Med. 54:785, 1959). There was no retraction defect using the method of Hartmann and Conley, as well as no evidence of fibrinolysis. When the platelet count was above normal the prothrombin consumption test always gave normal results. However, when the platelet count diminished or decreased to the normal level the consumption test became abnormal. Consistent with this the thromboplastic activity of platelets was found to be abnormal in all stages of this disease using the platelet thromboplastin generation test and platelet factor 3 assay. This platelet factor 3 activity deficiency was quantitative because no correction took place after disintegrating the platelets with sonic oscillator or distilled water. The red cell fallout phenomenon which is the inability of the blood clot to hold red cells, was abnormal in all cases. This abnormality continued after treatment although the hemoglobin level and the platelet count became normal and will be discussed in another presentation (supported by N.I.H. grant H- 3636 Ri).

2 *Physiology*

PERIPHERAL COUNTS AND SURVIVAL OF BLOOD PLATELETS OF MICE. T. T. Odell, Jr., and T. P. McDonald.* Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee.

Peripheral blood platelet counts were made on 92 BDF_1 male mice 11-22 weeks old by the direct phase microscope method. Average overall peripheral platelet count was 1.523×10^6 platelets/mm^3 ± 0.218×10^6 (1 standard deviation). To determine the period of time required to label the entire peripheral platelet mass with $Na_2S^{35}O_4$ in mice, labeled sulfate (1 μc/g) was injected each day intraperitoneally, and radioactivity of platelets was determined at daily intervals. To obtain sufficient platelets for radioassay it was necessary to kill animals serially. Platelets were collected from the blood, washed, resuspended in saline, counted numerically, and samples placed on planchets for counting with a windowless gas flow counter. Platelet radioactivity increased at a constant rate and reached its maximum 4-5 days after the first injection, suggesting that one complete replacement of the peripheral platelet mass takes place in this length of time. By this interpretation it follows that the average survival time of mouse platelets in the peripheral circulation is 4-5 days.

3 *Physiology*

THE EFFECTS OF CLOTTING ON PLATELET THROMBOPLASTIC ACTIVITY. Jessica H. Lewis. Univ. of Pittsburgh Sch. of Med., Pittsburgh.

Platelet thromboplastic or factor 3 activity is defined as that activity necessary to produce a normal clotting time and prothrombin consumption in thrombopenic blood or full prothrombinase (plasma thromboplastin) activity in the TGT test. Platelets were recovered from the clot of recalcified or thrombinized platelet rich plasma either by maceration or dissolution of the fibrin. Such "clotted platelets" isolated 60 minutes after the formation of the clot showed full factor 3 activity and variable but low complete prothrombinase action. Platelets isolated from recalcified plasma, citrated at intervals during clotting, showed an apparent decrease in platelet factor 3 activity at the time of beginning plasma coagulation followed by a return of this activity in the later samples. Coincident with this decrease in platelet factor 3 activity, there was an increase of full prothrombinase activity as evidenced by increased ability of the platelets to clot platelet poor plasma and other substrates.

XUM

4 *Physiology*

SURVIVAL OF PLATELETS TRANSFUSED INTO THROMBOCYTOPENIC RECIPIENTS. Dudley P. Jackson and Julius R. Krevans. Department of Medicine, Johns Hopkins Univ. and Hosp. Baltimore, Md.

Dogs were rendered thrombocytopenic by 500r whole body radiation. Blood from normal dogs was collected in plastic bags or uncoated glass bottles using ACD solution as the anticoagulant and was stored for varying periods. The blood was administered to irradiated dogs after the recipient's platelet levels decreased to <20,000/cu.mm. Exchange transfusion of 2,200 ml. of fresh blood collected in plastic bags raised a recipient's platelet level to 270,000/cu.mm. In contrast, exchange transfusion of 2,600 ml. of blood that had been stored in plastic bags for 10-13 days did not significantly increase a recipient's platelet level. Infusion, without exchange, of 500 ml. of fresh blood collected in uncoated glass bottles raised a recipient's platelet level to 160,000/cu.mm. Infusion, without exchange, of 1,000 ml. of blood collected in plastic bags and stored for 6 days did not elevate a recipient's circulating platelet level. Infusion of blood stored for 24-72 hours raised recipient platelet levels to 40,000-100,000/cu.mm. These studies in thrombocytopenic recipients indicate that survival of platelets contained in transfused blood is inversely related to the time of storage of the blood and is not dependent upon the use of non-wettable equipment.

5 *Pathology*

PURIFICATION AND ASSAY OF THROMBOPLASTINASE. A. Ottolenghi, S. Gollub and A.W. Ulin. (Intr. by S. Mudd). Sidney Hillman Medical Center, Philadelphia, Pa.

Methods of purification of the thromboplastinase (TPase) produced by a Bacillus megatherium-cereus intermediate have heretofore yielded preparations of doubtful purity. A method is given by which a maximum yield and high purity are achieved. One l. of brain heart infusion according to Gollub et al., Proc. Soc. Exp. Biol. & Med. 83, 858, 1953 was inoculated with B. cereus (xylose pos.) ATCC #10987 and incubated overnight at 37 C with moderate aeration. The centrifuged culture fluid was passed through a tricalcium phosphate column 4x3 cm. and the effluent discarded. Elution was performed with 0.5M Na citrate and the active eluate was dialyzed against distilled water. The dialysate was lyophilized and the enzyme stored as a powder. The column retained 5% of the culture fluid protein representing 99% of the enzyme activity. It was quantitatively recovered in the eluate. When human brain thromboplastin (TP) is incubated with TPase it loses its ability to clot recalcified plasma. An assay was developed by which a unit of TPase was defined as the amount necessary to destroy in 10 minutes ½ the TP which clots recalcified plasma in 55-57 seconds. At these concentrations linear relationships exist between TP and clotting times. A high degree of purity and a high activity were shown by physical and assay procedures.

6 *Pathology*

PLATELET ABNORMALTIES IN PERNICIOUS ANEMIA. J.M. Riddle*, J.W. Rebuck and R.W. Monto. Henry Ford Hosp. Detroit, Mich.

Electron microscopic studies of platelets from patients with P.A. in relapse and in remission revealed platelet ultrastructural abnormalties. Using Braunsteiner's heparin method five cases of untreated P.A. with moderate thrombocytopenia and poor prothrombin consumption were studied. Platelet differentials in the electron microscope revealed predominance of abnormal dendritic forms showing minimal pseudopodial formation (abortive dendritic forms). Studies of nine cases of P.A. treated with B_{12} and folic acid revealed poor or borderline prothrombin consumption in seven and normal values in but two. Eight cases showed persistence of abortive dendritic formation and eight presented increased viscous metamorphosis (VMs) with osmiophilic granulomere retention; in six cases spread forms without osmiophilic material were increased and two cases showed an increase in VMs without osmiophilic material. Sequential studies of a case of P.A. of Pregnancy revealed ultrastructural change from the untreated predominant abortive dendritic forms to a treated picture with a predominance of spread forms without osmiophilic material and increased VMs. These studies indicate that in addition to the persistent defect in red cell size and prothrombin production in P.A. in remission reported by Owren there is also a continuance of platelet abnormality.

7 *Pathology*

CATION REQUIREMENTS OF THROMBIN FOR PLATELET AGGLUTINATION. R.W. Shermer*, R.G. Mason*, R.H. Wagner* and K.M. Brinkhous. Dept. of Pathology, Univ. of North Carolina, Chapel Hill, N.C.

The activity of thrombin in causing platelet agglutination was studied, using a macroscopic test (Fed. Proc. 17;1749, 1958) previously described for a non-thrombic agglutinating factor (TAg) in canine plasma. Bovine, canine, and human thrombin preparations, diluted to a final concentration of 2 Iowa units/ml., were tested with canine and human platelets. Thrombin alone did not cause agglutination in this test. In the presence of certain divalent cations thrombin caused prompt agglutination (10-20 sec., 4 plus reaction). Of 4 divalent cations which were active with TAg, Mg and Mn were also active with thrombin, while Co and Ni were inactive. Mg and thrombin caused prompt agglutination of both canine and human platelets. Of the other ions used in this test, Ca was more active with human platelets than with canine platelets. On the other hand, Mn was more active with canine platelets. Species origin of platelets rather than source of thrombin seemed to determine differences in the relative effectiveness of the various ions in agglutination. High levels of cations were inhibitory. For comparable agglutinating effects, thrombin required a higher concentration of cations than did TAg. (Aided in part by Grant H-1648, NIH, PHS.)

033. BLOOD–Coagulation–Thrombin

1 *Physiology*

STUDIES ON A HEPARIN COFACTOR IN NORMAL AND IRRADIATED DOGS. Walter L. Milne* and A.K. Davis. U. S. Naval Radiological Defense Lab., San Francisco 24, Calif.

Heparin has been shown to produce marked changes in the clotting time of whole blood from irradiated animals. Since one of the known mechanisms of heparin-cofactor complex is to accelerate the inactivation of thrombin, experiments were designed to study the effect of added heparin on thrombin inactivation in an isolated plasma clotting system. The clotting time of oxalated, adsorbed plasma in the presence of graded amounts of heparin was determined after the addition of a standard amount of thrombin. After irradiation, heparin gradually loses its ability to accelerate thrombin inactivation. This effect is greatest at 17 - 21 days after irradiation, and is followed by a period of recovery toward normal values. Normal plasma which had been dialyzed against 0.9 per cent NaCl reacted to heparin in a manner similar to plasma from the irradiated animals. Furthermore, lyophilization of the dialysate and its addition to the irradiated plasma restored the clotting time to normal values. It is suggested that this dialysate represents a plasma cofactor of heparin which is reduced or altered after irradiation. The addition of this cofactor to the plasma of the irradiated dog produced normal clotting times, indicating that heparin is not present in excess of normal amounts after irradiation.

2 *Biochemistry*

ACTIVATION OF PROTHROMBIN, FACTORS VII AND X BY PROTEOLYTIC PATHWAY. Liberto Pechet* and Benjamin Alexander. Beth Israel Hospital, and Harvard University.

Earlier work on proteolytic prothrombin (pro) activation, suggested similarity to the thromboplastin (Tpl) pathway. Present availability of purified material derived by starch gel electrophoresis permitted study of the effect of proteolytic enzymes on separated components. Bovine fractions were exposed to trypsin and papain. Factors VII and X (Stuart) were assayed by the Owren and "Stypven" methods, and by the clot corrective effect on VII deficient patients. Trypsin produces thrombin directly from pro in the absence of VII and X, while simultaneously destroying evolved thrombin, confirming earlier work. The enzyme also rapidly activates VII and X, almost explosively at high concentrations. The effect is blocked by soy bean trypsin inhibitor (50 mcg. to 20 U. trypsin per ml.). Trypsin alone is inert in the assay system, and yields no thrombin from VII or X. Papain, also, activates VII and X but not pro in their absence, thus differing from trypsin. The similarity of proteolytic and Tpl activation was substantiated by the observation that VII and X in serum can be strongly activated by Tpl and Ca. The data support the concept that the biologic prothrombin activation system is fundamentally proteolytic. (Supported by a grant from Public Health Service, H-656.)

3 *Biochemistry*

SOME ASPECTS OF PROTHROMBIN ACTIVATION. Kent D. Miller, John McGarrahan*, and William H. Copeland*. Div. of Laboratories & Research, New York State Dept. of Health, Albany.

Since DFP does not inhibit the prothrombin activation by mixtures of calcium ion, tissue thromboplastin and serum accelerators, but does block the autocatalytic activation in sodium citrate (J. Biol. Chem. 223: 227, 1956), a basic difference between these activation mechanisms is suggested. The interesting activation of prothrombin by commercial protamine sulfate preparations described by Landaburu and Seegers (Am. J. Physiol. 193: 169, 1958) was also studied in this way. With prothrombin isolated by ion-exchange chromatography the phenomenon of protamine activation was substantiated. This activity of the protamine was not inhibited by 10^{-3} M DFP or by heating at 100°C. for 15 minutes. In mixtures of DFP with prothrombin and protamine, the prothrombin was consumed at a rate approximating that of thrombin formation in the absence of the DFP. Thus, with regard to DFP resistance, the protamine and bioactivation mechanisms are similar. Also, the protamine activation must depend either on an extraordinarily resistant enzyme or, quite possibly, on a nonenzymic reaction. Kinetic data are presented to characterize this type of prothrombin activation. Some properties of the thrombin produced by protamine activation are compared to those of thrombin derived from other activation procedures.

034. BLOOD–Erythrocytes

1 *Physiology*

THE HEMATOLOGICAL RESPONSE OF NEWBORN RATS TO X-IRRADIATION. George M. Cooper* and Albert S. Gordon. New York University and Sloan-Kettering Institute, N. Y. C.

Newborn rats were given a sublethal dose of X-rays (200 r) on their day of birth. Ten irradiated and ten non-irradiated animals were sacrificed at regular intervals after irradiation for hematological study. For comparison, the hematological response of a group of irradiated adult animals was also followed. The red cell counts, hemoglobin concentrations, and hematocrit values of the newborn animals fell sharply following irradiation to half the initial values by the tenth day, but recovered rapidly. On the other hand, the adult rats showed only small depressions in these values. The linear fall observed in the mean corpuscular volume of the large "fetal" red cells of newborn rats was retarded by X-irradiation, and is believed to be the result of an inhibiting action of irradiation on the formation of additional smaller red cells, which are probably of the adult variety. A post-irradiation leucopenia was observed in both adult and baby animals. It is concluded that the greater sensitivity to X-irradiation of the hematopoietic processes in newborn as compared to adult rats is a reflection of the rapid growth rate in the former which imposes a greater burden on these processes in the young than in the adult animal. (Supported in part by PHS grant H-3357.)

2 *Biochemistry*

THE SEPARATION OF ERYTHROCYTE ENZYMES FROM HEMOGLOBIN. M. A. Hennessey*, A. M. Haffner*, and B. W. Gabrio. Univ. of Wash., Dept. of Biochem. and King County Blood Bank, Seattle.

Investigations of the enzymology of the erythrocyte are limited owing to the fact that over 90% of the red cell protein is hemoglobin. A method has been devised to separate hemoglobin from enzymic proteins (EP) with the use of DEAE-cellulose. An hemolysate (pH 7.0) of washed human red cells was prepared by the freeze-thaw technique and mixed with DEAE at 4°. Hemoglobin was removed by washing the resin by centrifugation with 0.003M phosphate, pH 7.0. The EP fraction was desorbed by treatment of the resin with 0.5M phosphate, pH 7.0. In a typical experiment, approximately 2 g. EP-protein/100 ml. red cells are obtained. Specific enzymatic assays have revealed that the EP fraction contains the majority of the enzymes involved in glycolysis and the hexose monophosphate shunt pathway. Recovery of the activities of lactic dehydrogenase and G-6-P dehydrogenase was 95% and 85%, respectively. In addition, nucleoside phosphorylase, TPNH-methemoglobin reductase, and glutathione reductase activities can be recovered. A comparison of the relative activities of the enzymes in the EP fraction will be presented, and the advantage of using this hemoglobin-free fraction in the study of metabolic sequences in the red cell will be discussed.

3 *Biochemistry*

LINEAR GRADIENT ELUTION FROM DOWEX-1-FORMATE FOR NUCLEOTIDES AND PHOSPHATE ESTERS OF THE ERYTHROCYTE. S. A. Morell, V. E. Ayers* and T. J. Greenwalt.* Milwaukee Blood Center and Dept. of Biochemistry, Marquette Univ. School of Medicine, Milwaukee, Wis.

The use of extended convex gradient elution from Dowex -1-formate for the analysis of acid extracts of erythrocytes has been described (Willoughby and Waisman, Cancer Res. 17:942, 1957; Vanderheiden et al, Fed. Proc. 17:327, 1958; Mills and Summers, Arch. Biochem. and Biophys. 84:7, 1959). In studying the acid-soluble phosphates of erythrocytes, it was desirable to minimize the relatively high background absorption of the formic acid system of Hurlbert et al (J. Biol. Chem. 209:23, 1954). The discrete elution patterns of Bergkvist and Deutsch (Acta Chim. Scand. 8:187, 1954) served as a basis for an efficient single column linear gradient elution analysis of acid extracts of erythrocytes at low formate concentrations. An advantage of linear gradient elution is illustrated by the ease with which the data of Bartlett (J. Biol. Chem.: 234, 459, 1959) were applied to the successful resolution of 2,3-DPG and ATP. The apparatus, procedure and elution of reference compounds are described. (Supported by P.H.S. Grant RG5243)

4 *Biochemistry*

ELECTROMAGNETOPHORESIS OF ERYTHROCYTES. W. B. Dandliker, Wm. P. Murphy Jr.*, J. Walter Keller Jr.*. Miami Heart Institute, Miami Beach, Florida.

Kolin and co-workers (B.B.A., 32, 535, 1959) have shown that the force on a conductor in a magnetic field gives rise to the migration of particles suspended in a fluid if the phases differ in electrical conductivity. We have applied this principle to the separation of human erythrocytes from plasma. Erythrocytes migrate only very slowly at current densities of 0.25 amp cm^{-2} in a field of 24 kgauss (separation factor, S.F. about 1.3). The migration velocity can be greatly increased by causing the cells to aggregate somewhat either by the addition of isoagglutinins or of glycerol pectate which promotes rouleaux formation. The effect of aggregation is an expected one when Kolin's equation for the velocity of migration of spheres is considered. When the volume fraction of cells is about 0.05, virtually complete separation of cells and plasma (S.F. about 10) is obtained with a flow velocity of 50 cm min^{-1} at 2% glycerol pectate. The separation cell used is a molded polyvinyl chloride tube with a 0.02 cm^2 rectangular cross section, 6 cm long. To avoid remixing by electromagnetically engendered convection, a septum separates the fractions before they emerge from the magnetic field. (Supported by U. S. Air Force R&D Cont. AF41(657-194).

5 *Pharmacology*

EFFECT OF XANTHINES ON IN VITRO LYTIC ACTION OF MERCURIALS ON RABBIT ERYTHROCYTES. H. Minatoya*, F.P. Luduena and Leo von Euler*. Pharmacology Sect., Sterling-Winthrop Research Inst., Rensselaer, N. Y.

Theophylline reduces the irritancy of mercurial diuretic solutions (De Graff and Batterman, 1935). Using a method similar to that of Benesch and Benesch (1954) we found that theophylline also decreases the hemolytic activity of mersalyl. Thrice-washed erythrocytes were diluted with glucose free Tyrode solution making 1/20 standard suspension Using 5 cc. standard, the final 10 cc. samples were prepared and incubated at 37°C. for 1 hr. The concentration of mercurials was graded at 0.3 log intervals from 1/4 to 1/2000 mg.Hg/cc. The photoelectric colorimeter readings were expressed in % and the ED_{50} values (4-32 tests) were determined from linear dose-effect curves plotted on log-probit paper. Mercuric acetate was approximately 8 times as hemolytic as mersalyl. ED_{50} values of 1/900 and 1/115 mg.Hg/cc. were obtained with mercuric acetate and mersalyl, respectively. Addition of 4 mols of theophylline to 1 mol of mersalyl increased the ED_{50} of mersalyl from 1/115 to 1/15 mg.Hg/cc. Theobromine was much less effective.

XUM

6 *Pharmacology*

COMPARATIVE STUDY OF ERYTHROCYTE SODIUM AND POTASSIUM CONCENTRATIONS IN VERTEBRATES. Wayne S. Zundel* and Dixon M. Woodbury. Pharmacology Dept., Univ. of Utah, Salt Lake City.

In an attempt to determine if the high erythrocyte Na concentration characteristic of dogs and cats is limited to the Carnivora, a study was made of various orders of mammals and other vertebrates. Blood was obtained from animals in the field, and plasma and cells were separated immediately in a portable centrifuge. Na and K concentrations were estimated by flame photometry; red cell concentrations were calculated indirectly. The orders Chiroptera, Edentata, Primates, Rodentia, Lagomorpha, and Perissodactyla, and the classes Pisces, Amphibia, Reptilia, and Aves were found to have K as the major intracellular cation. Marked variability was found in the Artiodactyla. A high erythrocyte Na concentration was found throughout the Carnivora (families studied: Canidae, Procyonidae, Felidae, Mustelidae). These data suggest that the high intracellular Na concentration represents an evolutionary change toward loss of the Na transport mechanism by the membrane of the mature erythrocyte. In addition, the taxonomic distribution of this characteristic is compatible with the present concept of the evolutionary development of the mammals. (Supported by the Univ. of Utah Research Fund and Grant B-381, N.I.H.)

7 *Pathology*

AGGLUTINATION OF RAT ERYTHROCYTES BY SERUM FROM RATS TREATED WITH METHYL CELLULOSE. Donald A. Rowley and Frank W. Fitch. Dept. of Pathology, University of Chicago, Chicago, Illinois.

Rats receiving repeated intraperitoneal injections of methyl cellulose developed splenomegaly and hemolytic anemia (Giblett et al., Blood 11: 1118, 1956). Serum from methyl cellulose treated rats contains methyl cellulose; the serum agglutinates erythrocytes from normal or treated rats. "Agglutinin" titers are relatively high if normal rat serum is used as diluent and as a suspending medium for erythrocytes; "agglutinin" titers are markedly inhibited if saline is used as diluent and as a suspending medium. When methyl cellulose is added to normal rat sera in concentrations equivalent to that found in sera from methyl cellulose treated rats, similar "agglutinin" titers are found. The anemia produced by methyl cellulose is prevented by splenectomy. "Agglutinin" titers of sera from splenectomized and non-splenectomized methyl cellulose treated rats will be compared. It has been suggested that methyl cellulose by inducing hyperfunction of the spleen produces hemolytic anemia. Our findings suggest that splenomegaly and anemia are the result of circulating methyl cellulose and the agglutination of erythrocytes it produces.

8 *Pathology*

ERYTHROPOIETIC INHIBITING FACTOR IN RED BLOOD CELLS. Blunt, J. Wallace and Berg, Marvin*. St. Luke's Hospital, Cleveland, Ohio.

If an erythropoietic stimulating factor is constantly present in plasma it was postulated that an erythropoietic inhibiting factor should be present within the RBC to be released on destruction of the cell to maintain a balance between E.S.F. and E.I.F. Autogenous and homogenous, dog, washed RBC were lysed by H_2O, by freezing-thawing and by ultra-sound. I^{131} T.B.V., R.C.V., P.V., Cr^{51} RBC survival times, Fe^{59} plasma clearances and Fe^{59} RBC uptakes were done on normals and after I.V. injections of hemolysed RBC. By giving lysed RBC equal to 3 times the expected volume of destroyed RBC the dogs developed anemia in 3 weeks. By giving the same amount daily to dogs bled 40% of T.B.V., the R.C.V. decreased further; some animals dying in cardiac failure. The Fe^{59} RBC uptake during injection period showed minimal erythropoiesis. When the I.V. hemolysate was stopped a few animals developed transient, marked polycythemia. No change in kidney function or morphology was noted. (Supported by The Elisabeth Severance Prentiss Foundation).

9 *Immunology*

ANTIGENIC PROPERTIES OF BOVINE RED CELL STROMA. Becca Patras and W. H. Stone. (intr. by M. R. Irwin.) Genetics Dept., Univ. of Wis., Madison, Wis.

The ultimate aim is to study the chemical nature of specific blood group substances on cattle erythrocytes. To obtain stromata, one volume of washed, packed cells was added to ten volumes of either distilled water or 0.01 M citrate buffer pH 5.6. This suspension was centrifuged at low speed to remove large particles (RI). The supernatant was adjusted to pH 4.7 (acid ppt.) or was centrifuged directly at 25,000 x g for 40 minutes (RII). Further centrifugation of RII supernatant at 105,000 x g for 15 minutes produced no further sediment. Acidification of the RII supernatant to pH 4.7 resulted in a third precipitate (RIII). The specific antigenic activities of these preparations were investigated using absorption and hemolysis inhibition. Preparation RII exhibited the greatest total activity and the highest specific activity (units/mg. protein). RIII had low activity for several blood factors, notably F and V. The effects of some chemical agents on blood group activity of the RII preparation indicated that a study of blood group substance of red cell stroma is feasible. (Supported in part by Grant No. E-1643(C5) from Natl. Insts. of Health, P.H.S.)

035. BLOOD—Erythrocytes—Destruction

1 *Physiology*

THE ROLE OF HAPTOGLOBIN IN THE CLEARANCE OF EXTRACORPUSCULAR HEMOGLOBIN. R.K.Murray, G.E.Connell, and J.H.Pert, Dept. of Biochemistry, University of Toronto, Canada. (intr. by K. C. Fisher.)

Hemoglobin labelled with Fe^{59} was injected intravenously into rabbits and the clearance from plasma was observed. In these animals the plasma haptoglobin levels were low relative to the quantity of hemoglobin injected. The average time for clearance of half the hemoglobin was 24 minutes. Two hours after injection 40% of the radioactivity was found in the kidneys and the remainder was distributed principally between liver, spleen and bone marrow. In other experiments labelled hemoglobin bound to haptoglobin was injected and here the average time for half-clearance was 88 minutes. Four hours after injection most of the radioactivity was found in the liver, with only traces in the kidney. These experiments confirm the findings of Laurell and Nyman (Blood, 12, 493, 1957) in humans that hemoglobin passes freely into the glomerular filtrate while the hemoglobin-haptoglobin complex does not. The clearance studies with hemoglobin and the complex were repeated using nephrectomized animals. Although the clearance rates in both cases were slower than in the normal animals, the rate of clearance of free hemoglobin was still significantly faster than that of the complex.
(Supported by the National Research Council of Canada.)

2 *Biochemistry*

RELATIONSHIP BETWEEN GLUTATHIONE CONTENT OF RAT ERYTHROCYTES AND HEMOLYSIS BY VARIOUS AGENTS IN VITRO. H. B. Collier and C. C. Tsen*. Dept. of Biochemistry, Univ. of Alberta, Edmonton, Canada.

Erythrocytes (RBC) from tocopherol-deficient and supplemented rats had identical levels of free glutathione (GSH) as determined by the alloxan "305" method; yet only the tocopherol-deficient RBC were hemolyzed by dialuric acid or by shaking in an atmosphere of oxygen. This treatment decreased the GSH in both groups of cells, but the extent of hemolysis did not parallel this decrease. Treatment with selenite, iodoacetate, or N-ethylmaleimide reduced the red-cell GSH to very low levels but there was little hemolysis. Silver nitrate, mercuric chloride, or p-chloromercuribenzoate were powerful hemolytic agents, but did not decrease the free GSH of the RBC except when present in high concentrations. (In these experiments there were no significant differences between the tocopherol-deficient and supplemented RBC.) Conclusion: susceptibility to hemolysis, under the conditions of our experiments, is not related to the levels of free GSH. The heavy metals probably act directly upon the RBC membrane. (Supported by grants from the Defence Research Board of Canada, Project D50-9350-06, the National Research Council of Canada, and a NRC Postdoctorate Fellowship to C.C.T.)

3 *Immunology*

ROLE OF MEDIUM COMPOSITION AND COOLING RATE IN FREEZING AND THAWING RED CELLS. G. F. Doebbler, R. E. Greco, and A. P. Rinfret (Intr. by E. Witebsky) Linde Company, Division of Union Carbide Corporation, Tonawanda, New York

Recoveries of human red cells after freezing and thawing in synthetic media of various compositions have been examined with respect to (1) electrolyte concentrations and nature of ions present, (2) concentrations and types of protective additives, and (3) cooling rates. Extracellular but not intracellular salt concentrations affect recovery. Decreasing recoveries are obtained in the presence of the anions Cl, Br, and SCN, but not the cations Li, Na, and K. At about equal weight concentrations polyvinylpyrrolidone (cells impermeable) and glycerol (cells permeable) give similar protection at cooling rates up to 3°C/sec. Increase in cooling rate from about 0.3 to 3.0°/sec. usually results in increased cell recoveries and improvement in the level of protection afforded by a given additive concentration. With rapid cooling (about 30°/sec.) high recoveries are favored in hypertonic media of high salt concentration although excessive hypertonicity appears undesirable. With slow cooling high additive concentrations are required for high recoveries regardless of salt concentration or medium tonicity.

036. BLOOD–Erythrocytes–Formation

1 *Physiology*

RAT ASSAY OF ERYTHROPOIETIC FACTOR IN MILK OF "ALTITUDE" GOATS. J. Clifford Stickney, Theadore L. Browne* and Edward J. Van Liere. West Virginia Univ. Med. Ctr., Morgantown.

Milk obtained daily immediately after 4 hr exposure of 3 goats to 22,000 ft was frozen and stored. The whey from the thawed fat-free milk was dialyzed against distilled water to remove lactose. Whey proteins were precipitated by acidifying to pH 5.0 and heating to boiling; filtering, washing ppt with water and lyophilization of the filtrate followed. Control milk obtained from the 3 goats when not exposed to altitude was treated likewise. Four-tenths g of the two extracts or 1 ml saline were injected ip into groups of 5 female (290 g) Wistar albino rats for 5 days. Reticulocytes on tail tip blood were determined on wet smears stained with Brilliant Cresyl Blue and Wright's stain 6 days prior to injections, on the 5th day of injections and 1 wk later. Pre-injection values were alike in the 3 groups. Fifth day reticulocytes were 1.78 and 3.01% (p:0.006) for control and altitude milk groups respectively; 12th day: 1.89 and 3.37% (p: 0.002). Fifth and 12th day values for saline controls were 1.19 and 1.49%. It is concluded that the erythropoietic factor in the milk of "altitude" goats is effective in the rat. (This investigation was supported by research grant RG-5149 from Division of General Medical Sciences, Public Health Service)

2 *Physiology*

A POSSIBLE ERYTHROPOIETIC ACTION OF CHOLINESTERASE. John Emerson Davis, Coll. of Pharmacy, University of Texas, Austin.

Plasma erythropoietic stimulating factor (ESF) is increased in animals by cobalt administration, or by the production of a marked anemia with phenylhydrazine. We have found that these two procedures also cause a modest but consistent increase in plasma cholinesterase activity. This, together with other evidence, suggests the possibility that cholinesterase or part of its molecule may be concerned with erythropoiesis. Normal rats weighing about 180 gm were injected subcutaneously with acetylcholinesterase (concentrate from bovine erythrocytes) in doses of 4 mg (200 units) daily. On the fifth experimental day, reticulocyte percentages had increased from an average control value of 1.2 up to 7 (range 4.4 to 9.5). Within 15 days, erythrocyte counts showed an average increase of 1.7 million (or 22%); but hematocrit percentage of packed cells was practically unchanged. Untreated control animals showed no reticulocytosis or erythrocytosis during the experimental period. It is concluded that acetylcholinesterase exerted an early erythropoietic stimulating action in normal rats. This effect was not well maintained, however, since the erythrocytosis was less marked toward the end of the 18-day injection period.

3 *Physiology*

SIMPLIFIED KINETIC DESCRIPTION OF RBC SYSTEM. J. E. Roberts* and T. H. Allen. Univ. of Colo. School of Med., Denver, Colo. and USA Med. Res. & Nutr. Lab., Fitzsimons Army Hosp., Denver, Colo.

The production of r.b.c.'s is assumed to commence with self-perpetuating stem cells and to proceed through six types of cells until destruction of the final cell type occurs. The numbers of cells of each type are connected by a system of first order, linear differential eq's terminating with a Gaussian survival function. Accepting three duplicating generations and knowing the total number of circulating r.b.c.'s, N_7, and a mean survival time, $\bar{T}$, the flux of stem cells is $N_7/8\bar{T}$ in a steady state. Between two steady states the ratio of the corresponding stem cell rate parameters and their progeny can be ascertained by noting the ratios of erythrocytic cell types to granulocytes in a sample of marrow, provided the body's total number of granulocytes is constant. Referring to reports from the Andean Inst. of Biology: whereas the stem cell flux is almost doubled by long residence at 14,900 ft. elevation, some of the subsequent rate parameters are reduced to one-half of the sea level values, implying a delayed maturing of some daughter cells.

4 *Physiology*

Fe^{59}DTPA DISTRIBUTION AND EXCRETION IN NORMAL AND ANEMIC RATS. J.V.Principotto, R.Daly* and M.Rubin*. Dept. Physiology and Biophysics, Georgetown Univ. School of Med., Washington, D.C.

The synthetic amino acid chelates have recently been under extensive investigation because of their value as replacements or supplements to the natural protein chelates which function as metal transporting agents in the body. The nature of the organic carrier of a metal may markedly influence its absorption, distribution, and excretion in vivo. Diethylenetriaminopenta-acetic acid (DTPA), an analog of EDTA, seems to have many of the characteristics of the plasma iron-transport protein, and, because of its smaller molecular size, it has added advantages. Iron-DTPA (6.0 mg/kg) injected i.v. into anemic rats resulted in approximately a 25% iron uptake by the RBC and a 70% urinary iron excretion in a 24 hr. period. Over 95% of the urinary iron-chelate excretion occurred in the first 6 hours. When Iron-DTPA was given in the same manner to normal rats, the RBC iron uptake was 5% and the urinary iron excretion was 90%. DTPA seems to carry iron in the body in such a way that it is released for erythrocyte formation as needed. The iron not utilized for erythropoiesis is excreted in the urine as the iron chelate. These experiments suggest a new approach to chemotherapy of iron deficiency and of iron storage disease.

5 *Biochemistry*

SUPPRESSION OF ERYTHROPOIESIS BY CERTAIN ESTROGENS AND THIOCYANATE. Peter P. Dukes* and Eugene Goldwasser, Argonne Cancer Res. Hosp.[a] and Dept. of Biochemistry, University of Chicago, Chicago, Ill.

The rate of erythropoiesis in rats, when measured by incorporation of Fe^{59} into red cells, can be stimulated by erythropoietin (E) (exogenous) or by $CoCl_2$ or hypoxia (endogenous E). Depression of erythropoiesis can be demonstrated following administration of diethyl stilbesterol, hexesterol, estradiol, estriol and some related compounds. Testosterone, progesterone, and cortisol have no suppressive effect while NaCNS inhibits red cell formation. Stilbesterol and estradiol can reduce the effect of either endogenous or exogenous E. Maximal suppression occurs with stilbesterol when injected 3 days prior to Fe^{59} administration while no effect is seen when the two are given simultaneously. We suggest that E and these antagonists competively displace each other during an early event in the sequence leading to the formation of mature red blood cells.

[a]Operated by The University of Chicago for the United States Atomic Energy Commission.

XUM

037. BLOOD–Erythrocytes–Metabolism

6 *Pathology*

ERYTHROCYTE DELTA AMINOLEVULINIC ACID DEHYDRASE: A SENSITIVE INDEX OF HEMOGLOBIN SYNTHESIS. J.H.Sacks*, L.A.Mounter and R.H.Egdahl, Surg. Res. Lab., Med. Coll. Va., Richmond, Va.

Delta aminolevulinic acid dehydrase (ALAD) is necessary for the enzymatic condensation of two molecules of ALA into porphobilinogen, and therefore ultimately for the synthesis of porphyrins and heme. It appeared that ALAD activity in erythrocytes might reflect quantitatively the synthesis of heme of hemoglobin. During the phase of hemoglobin regeneration following the induction of an acute hemolytic episode in rats, animals were sacrificed daily and ALAD activities, blood hemoglobin concentrations and reticulocyte counts were determined. ALAD activity was determined by an estimation of the amount of porphobilinogen formed during the anaerobic incubation of the activated enzyme with ALA. It was found that: 1) ALAD activity is not measurable in the normal rat. 2) There is a linear relationship between erythrocyte ALAD activity and hemoglobin synthesis in animals regenerating hemoglobin. 3) Reticulocyte counts correlate poorly with both hemoglobin synthesis and ALAD activities. Erythrocyte ALAD activity appears to be a sensitive index of hemoglobin synthesis.

7 *Pathology*

ERYTHROPOIETIC STIMULATING FACTOR (ESF) PRODUCTION IN NEPHRECTOMIZED RABBITS. Neil I. Gallagher, John M. McCarthy and Robert D. Lange (intr. by W. J. Harrington.) VA Hospital, St. Louis, Missouri.

Extracts of plasmas from normal rabbits, hypoxic intact rabbits, nephrectomized rabbits and hypoxic-nephrectomized rabbits were bio-assayed for ESF content. Hypoxia was induced in a low atmosphere chamber for eight hours immediately prior to sacrifice. The extracts were bio-assayed in Sprague-Dawley rats rendered polycythemic by transfusion. The 18-hour red cell radioiron incorporation in the assay rat was the parameter used to measure ESF content of the various plasma extracts. Polycythemia reduced Fe^{59} incorporation from the normal 30% 4.0% (SDM) to 6.0% 1.06% (SDM) in the assay rat while injection of the assay animals with hypoxic plasma extracts increased incorporation from 6.0% to 23.5% 4.2%. Polycythemic rats injected with plasma from hypoxic nephrectomized rabbits consistently incorporated more Fe^{59} (9.4% 1.6%) than those injected with normal plasma extracts (5.2% 0.8%) or plasma extracts from nephrectomized non-hypoxic rabbits (4.9% 1%). These experiments demonstrate that the nephrectomized rabbit retains a limited but consistent ability to produce ESF in response to hypoxia.

8 *Nutrition*

ERYTHROPOIESIS IN DUCKS WITH DIFFERENT B VITAMIN DEFICIENCIES. Dan A. Richert, B. Pixley*, and M. P. Schulman*. Dept. of Biochem., State Univ. of N. Y. Med. Coll., Syracuse.

The purpose of this study was to see whether heme synthesis in vitro was retarded in red cells from ducks deficient in niacin, riboflavin, thiamine, pantothenic acid or vitamin B_6. Since the synthesis of heme from glycine takes place in immature erythrocytes and since the reticulocyte counts were decreased in the bloods of the deficient ducks, a reticulocytosis was induced by phenylhydrazine to provide more favorable conditions for studying the rates of heme synthesis. By comparison with controls also treated with phenylhydrazine, the reticulocyte counts (%) were as high or higher in the deficient bloods; hemoglobin concentrations, hematocrits and total erythrocyte counts were lower; except in vitamin B_6 deficiency, the mean cell volumes and mean cell hemoglobin concentrations were in the control range. Except in vitamin B_6 deficiency, the rates of glycine uptake into heme were in the same range as for treated or untreated controls. Thus it appears that the basic hematologic defect was an inability to form enough cells to compensate for the destruction by the drug, and the anemias were not (except in vitamin B_6 deficiency) attributable to retarded heme synthesis per se. (Aided by grants NSF G-7126 and PHS C-1852.)

1 *Physiology*

PHOSPHORYLASE ACTIVITY IN HUMAN ERYTHROCYTES. M. Cornblath, E.Y. Levin, E. Marquetti, and E.Y. House (intr. by C. Cohn) Michael Reese Hospital, Chicago and Sinai Hospital, Baltimore.

Phosphorylase has not been assayed in human red cells before and the characteristics of this reaction are defined. In the cold, red cell hemolysate was prepared by washing cells with .08M NaF-.08M NaCl, suspending in equal volume of NaF-NaCl, adding NaF to .18M, incubating 5' at 37°C and quick freezing. 2cc hemolysate was incubated 4 hrs. at 37°C with 10μM G-1-P and 1.2% glycogen. Glycogen synthesized was estimated by iodine color of TCA supernate and quantified by anthrone reaction of isolated glycogen.

UDPG, G-6-P, glucose or F-1-6 diphosphate instead of G-1-P abolished activity as did heating to 65°C for 10' or using old bank blood. Reaction was glycogen primer dependent except in 2 Glycogen Storage Disease hemolysates containing 2-10 mgm glycogen/gm Hb. Glycogen break-down was demonstrated incubating hemolysate with glycogen in PO_4 buffer.

Mean activity (range) of 24 assays of adult hemolysates was 44 mg glycogen/gm Hb/hr (25-75) as measured in TCA supernate; whereas by isolated glycogen and anthrone, the mean of 8 was 16 (15-32) mg/gm Hb/hr. In G.S.D. hemolysates, mean activity in 3 assays was 95 mg/gm Hb/hr in 1 and 39 mg/gm Hb/hr in the other. Glycogen break-down was similar in G.S.D. and controls. (Supported by a grant from N.I.H., U.S.P.H.S.)

2 *Physiology*

CA^{45} UPTAKE BY STORED DOG ERYTHROCYTES IN SODIUM AND POTASSIUM CHLORIDE SOLUTIONS. A. Omachi and R.P. Markel*. Univ. of Illinois College of Med., Chicago, Ill.

Washed erythrocytes prepared from heparinized blood were suspended in buffered 0.16M NaCl or KCl and shaken gently at room temperature for 30 minutes. Ca uptake was estimated from the disappearance of Ca^{45} from the medium. The uptake of Ca by fresh cells in NaCl and KCl was essentially similar. In contrast, cells stored in a refrigerator for 24 hours or longer took up more Ca in KCl but not in NaCl; e.g., at 48 hours 0.08 μM disappeared from the KCl solution compared to 0.02 μM from the NaCl medium per ml of cells. The addition of glucose to stored blood failed to affect these observations. Cells stored in acid-citrate-dextrose for as long as 5 days behaved like fresh erythrocytes. Slightly greater hemolysis was observed in KCl but fresh cells hemolyzed by hypotonic NaCl solutions failed to show uptake of the magnitude seen in aged cells suspended in KCl. These findings indicate (1) that stored erythrocytes take up more Ca in KCl solutions than fresh cells and (2) that the uptake by stored cells is greater in KCl than in NaCl. The latter may be due to competition of Na and Ca ions for binding sites as suggested in frog heart experiments (Niedergerke, Experentia 15; 12, 1959).

3 *Biochemistry*

METABOLISM OF INOSINE BY HUMAN RED BLOOD CELLS. Giovanni Bucolo * and Grant R. Bartlett. Scripps Clinic, La Jolla, Cal.

Human blood freshly drawn into heparin was incubated at 38° with inosine (15 μM/ml) and inorganic phosphate (40 μM/ml) and the water soluble phosphate compounds of the red cells were analyzed by ion-exchange chromatography at 1, 2, and 4 hours. Values are expressed in μM per ml of red cells. In 2 hours 20 μM of the ribose from inosine disappeared, 20 μM of lactate was formed and 25 μM of inorganic phosphate was esterified. There were several changes of interest in the pattern of phosphorylated intermediates. The concentration of ATP increased to 1.5 μM, 1½ times the normal value. AMP and ADP decreased and an equivalent amount of inosinic acid appeared. The level of 2,3-diphosphoglycerate rose about 50% to 7 μM. There was also a small rise in the monophosphoglycerate. Little change took place in the glucose diphosphate but fructose diphosphate increased several fold to 8.5 μM and this accounted for a large per cent of the metabolized ribose carbon not converted to lactate. Total sugar monophosphates rose from about 0.2 to 0.6 μM. Most of this increase was due to ribose 5-phosphate, dioxyacetone phosphate and an unidentified ketose phosphate. Only traces of sedoheptulose, ribulose and xylulose monophosphates were present. The addition of inorganic phosphate during inosine utilization greatly influenced the concentrations of the intermediates.

4 *Biochemistry*

HEME SYNTHESIS BY DUCK ERYTHROCYTES AS A FUNCTION OF AGE. J. Raymond Klein. Biology Dept., Brookhaven National Laboratory, Upton, N. Y.

During the first several days after whole-body X-irradiation (800-1200 r), the synthesis of heme by intact duck erythrocytes in plasma in vitro decreases to a low level. The formation of heme from iron and protoporphyrin by extracts of the cells and of free porphyrin by intact cells in the presence and absence of added glycine do not change. The irradiation produces an aging population of erythrocytes. Therefore, the decrease in heme formation represents a loss of function with increase in age of the circulating erythrocyte. The decrease in heme production implies a corresponding decrease in porphyrin synthesis which is apparently attributable to a loss in the capacity of the cell to obtain glycine. Porphyrin, but not heme, synthesis is restored by addition of glycine to the blood. The transfer of iron from plasma to the cell decreases after the irradiation and is not affected by adding iron to the blood. Therefore, the decrease in heme formation with age of the red cell is attributable to a decrease in activity of the cell system involved in obtaining iron from plasma. (Supported by USAEC.)

5 *Biochemistry*

AMYLASE IN DOG BLOOD. Michael Somogyi and Anne Dowdy*. The Jewish Hospital of St. Louis, St. Louis, Mo.

In assaying the amylase content of dog blood with Somogyi's two methods (J. Biol. Chem. 125:399, 1938), we came upon two essential differences as compared with human blood. In the first place, it is from 10 to 20 times greater; secondly, a substantial quota of the reducing matter that is formed from starch or glycogen consists of glucose. This fact led to the assumption that - unlike in human blood - it is not amylase alone, but a multi-enzyme system that accounts for the cleavage of starch or glycogen. This was actually found to be the case. Amylase was removed by a method described in the paper cited above, with the result that the amyloclastic (dextrinizing) action of the blood plasma was completely abolished, whereas the saccharogenic action was partially retained, the reducing matter formed consisting entirely of glucose, with exclusion of intermediary oligosaccharides. (No further studies were made for the identification of this saccharifying enzyme.) This fact would have escaped observation if the amylase action had been determined only by the method based on the saccharogenic action of amylase, and the formation of all of the reducing matter would have been attributed to amylase action.

038. BLOOD–Groups

Biochemistry

DETECTION OF RED CELL AGGLUTINOGENS IN CHICKENS BY LECTINS. S. L. Scheinberg and R. P. Reckel (intr. by N. R. Ellis.) Animal Husbandry Research Division, A.R.S., U. S. Dept. of Agriculture, Beltsville, Md.

The use of lectins as substitutes for antibodies, immune or naturally occurring, has become widespread in the typing of human red cells. Lectins for typing chicken red cells are particularly appropriate since much effort is spent in producing typing sera. Studies were undertaken to discover sources of agglutinins among a large array of plant seed extracts. The 64 extracts tested could be grouped as follows: 69% which did not react with the red cells of any of the chickens tested; 9% which agglutinated the red cells of all the chickens; 5% which hemolyzed the red cells of all the chickens and 17% which detected differences among individual chickens. Half of the extracts which detect cellular differences among the chickens are restricted in their reactivity to the red cells of females. Two of the latter extracts are Lathyrus cicera and Pisum arvense which are identical in their reactivity with chicken red cells. The agglutinogen appears to be associated with egg production, since there are changes in agglutination score with the rise and fall of egg production. The agglutinogen is best inhibited by D-Mannose and the agglutinin activity is easily isolated by salting out with ammonium sulfate.

2 *Immunology*

THE PURIFICATION OF BLOOD GROUP SUBSTANCES BY CHROMATOGRAPHY OF PSEUDOMUCINOUS OVARIAN CYST FLUIDS AND SALIVA. Harold Baer and Ursula Rasmussen.* Dept. Microbiology, Tulane Univ. School of Med., New Orleans, La.

Human pseudomucinous ovarian cyst fluid and saliva were subjected to chromatographic separation on DEAE cellulose columns. Blood group activity was found in several different fractions, in each case associated with fucose and an absorption peak at 280 mμ. This lends additional support to the hypothesis that blood group substances are a polysaccharide-protein complex and provides the unexpected concept that blood group activity may not be associated with a single molecular species. (Supported by a grant from the U. S. P. H. S.)

3 *Immunology*

HETEROGENEITY OF Rh_o(D) ANTIGEN. S.P. Masouredis. Dept. Prev. Med. & Cancer Res. Inst., Univ. Calif., San Francisco, Calif.

The Rh_o(D) red cell antigen was studied using an incomplete I-131 anti-Rh_o(D). The antibody was obtained from a 30 yr. female, A_2 rh (cde), who had been immunized to Rh_o(D) by 8 pregnancies and had been boosted by an I.V. injection of A_1, Rh_o (cDe) blood. The gamma globulins were isolated from her serum with cold ethanol techniques and trace labeled with I-131. The I-131 anti-Rh_o(D) was obtained by sensitizing Rh_o(D) positive stroma and then eluting the I-131 anti-Rh_o(D) from the stroma. In a population of 500 different red cells positive to Rh_o(D) by commercial antisera, 4 different cells were found that did not react with the I-131 anti-Rh_o(D). Two of the 4 red cells were D^u positive (O, $r^{u}r$-cdE/cde and O, $r'r$-cDe/cde) and 2 were D^u negative (B, R^or-cDe/cde or R^oR^o-cDe/cDe and O, R^or-cDe/cde or R^oR^o-cDe/cDe). The quantity of antibody nitrogen bound to these cells was less than 1% of the N bound to Rh_o(D) positive red cells. The specificity of this anti-Rh_o(D) was defined by the two exposures of the donor to the Rh_o(D) antigen and by the Rh_o(D) present in the stroma used for elution. These results show that the Rh_o(D) antigen is immunochemically heterogeneous and that quantitative evaluation of this variability can be achieved through the use of an I-131 labeled antibody. (Supported by U.S.P.H.S. Grant H-5071.)

4 *Immunology*

IDENTIFICATION OF ERYTHROCYTE BLOOD GROUP ANTIGENS, AND THE DEMONSTRATION OF MINOR CELL POPULATIONS WITH FLUORESCENT ANTIBODIES. F. Cohen, W.W. Zuelzer, and M. Evans (intr. by C.S. Stulberg). Child Research Center of Michigan, and Wayne State University College of Medicine, Detroit.

The demonstration of blood group antigens in erythrocytes by specific fluorescence was achieved by treating wet preparations of red cells with fluorescein isothiocyanate-labeled antibodies. Grp.A and Grp.B antigens were demonstrated by the direct method using coupled human anti A and anti B sera. A variety of Rh antigens and Kidd were demonstrated by the indirect method, in which red cells were sensitized with their specific incomplete antibody and then treated with labeled rabbit anti human globulin. Relatively low intensity of fluorescence with these weaker antigens was nevertheless specific since it could be shown that under the conditions employed, green fluorescence was an all-or-none reaction and various suitable controls showed no fluorescence at all. The method has been used to identify, and within reasonable limits, to quantitate minor cell populations in artificial mixtures (e.g. Grp.A cells in Grp.O cells, Rh+ cells in Rh- cells), in ratios up to 1:100,000. Injected Rh+ erythrocytes were demonstrated in the blood of an Rh- volunteer for as long as 3 weeks. Fetal Rh+ red cells were demonstrated in several Rh- women with and without antibody, in the last trimester of pregnancy. The method is being used to study mechanisms of isoimmunization.

XUM

5 *Immunology*

QUANTITATIVE HEMAGGLUTINATION STUDIES IN THE Rh SYSTEM. Robert Silber (intr. by F.M. Bozeman.) Walter Reed Army Institute of Research, Washington, D.C.

The quantitative hemagglutination technique of Wilkie and Becker (J. Immun. 74:192, 1955) has been adapted to the study of the Rh_0 (D) antigen and anti-Rh_0 (anti-D) antibodies. Incubation of the cell-antiserum mixture was carried out at 37° for three hours. The reversibility of this system has been established. By means of this technique, the saline anti-Rh_0 (anti-D) agglutinin has been investigated; it has also been possible to study the incomplete antibody by using cells treated with trypsin. A linear regression line is obtained when the percentage of cells agglutinated at equilibrium is transformed to probits and plotted against the logarithm of antiserum concentration. Various Rh cell types have been assayed with a standard antiserum and different antisera have been tested against standard cells. When examined with a given antiserum, cells within an Rh phenotype yielded log-probit regression lines which had the same slope but differed in position (HD_{50}), while cells of different Rh phenotypes tested with the same antiserum varied not only in HD_{50} but also in slope. Variations in HD_{50} and in slope were obtained when the same cell was tested against different antisera.

6 *Immunology*

A CHANGE IN CHARACTER OF THE ANTI-A ISO-ANTIBODY FOLLOWING IMMUNIZATION WITH BLOOD GROUP A SUBSTANCE. Mary B. Gibbs (intr. by A.S. Benenson.) Walter Reed Army Institute of Research, Washington, D.C.

With quantitative hemagglutination inhibition methods, it has been shown (Gibbs, M.B., Fed. Proc. 18:569, 1959) that the naturally occurring anti-A and the iso-agglutinin stimulated by blood group A substance differ in their reactions with hog A substance. Further studies of antisera obtained before and during immunization of humans injected with hog A substance have shown a transition in antibody behaviour from that characteristic of the naturally occurring anti-A to that observed with the stimulated iso-agglutinin. Concomitant with the rise in anti-A titer, the rate of dissociation of the substance-antibody complexes decreases, the slope of the log-probit regression line gradually becomes steeper, and progressively smaller quantities of homologous A substance are required to inhibit a standard hemagglutinating unit of antibody. This quantity is identical for the iso-agglutinins from different individuals stimulated with the same group A substance, when a constant slope has been attained.

7 *Immunology*

M AND N AGGLUTININ INHIBITION BY HUMAN KIDNEY AND ERYTHROCYTE EXTRACTS. Karlheinz Stalder* and Georg F. Springer, William Pepper Lab., Univ. of Pennsylvania, Philadelphia.

Information on the chemical nature of M and N erythrocyte agglutinogens has recently been obtained by enzymatic (Springer and Ansell, Proc. Nat. Acad. Sci. 44, 182, 1958) and chemical methods (Hohorst, Z.f. Hygiene 139, 561, 1954; Baranowski et al., Arch. Immunologii i Terapii Doświadczalnej 7, 15, 1959). The present report deals with the extraction of the M and N active principles from kidney medulla and erythrocyte stroma. Activity of extracted materials was determined with human anti-M sera and anti-N agglutinins from Vicia graminea. Most of the M or N active preparations inhibit other blood group agglutinins. Of several methods tried, extraction of the kidney medulla with 60% ethanol and subsequent fractionation with ether and water yielded highly active material in the ether-alcohol layer. Hot phenol-water extraction was as efficient as the alcohol-ether procedure when used on erythrocyte stroma but less satisfactory when used on kidney medulla. For the phenol extraction it was necessary to suspend the materials in physiological saline as only traces of activity were obtained when using a suspension in water. (Supported by the N.I.A.I.D., Natl. Inst. of Health.)

8 *Immunology*

ACQUISITION OF BLOOD-GROUP B-LIKE BACTERIAL ANTIGENS BY HUMAN A AND O ERYTHROCYTES. Georg F. Springer and Norma J. Ansell*, William Pepper Lab., Univ. of Pennsylvania, Philadelphia.

Smooth E. coli O_{86} is related serologically and chemically to human blood-group B antigen (J. Immunol. 76, 399, 1956; Fed. Proc. 18, 2375, 1959). Acquisition of B-like antigens by the erythrocytes of surgical patients has been described by numerous authors (Brit. Med. J., July-August, 1959), of whom F. Stratton, P. H. Renton and C. Cameron consider bacterial polysaccharides a possible source of these antigens. Soluble blood-group B active materials have now been found in culture media in the declining phase of growth of S. berkeley O_{43}, Arizona O_{21}, E. coli O_4, E. coli O_8, E. coli O_{55}, Proteus mirabilis, bacteria of Citrobacter and intermediate coliform groups and strains of E. coli O_{86}. These materials and purified lipopolysaccharides and protein-lipopolysaccharides from E. coli O_{86} coat human erythrocytes of all age groups making them agglutinable by anti-B antibodies. There is no apparent influence on agglutinability of preexisting blood group receptors. Anti-B agglutinins from A and O persons can be adsorbed onto E. coli O_{86} polysaccharide-coated A and O erythrocytes and anti-B specific agglutinins can be eluted. Erythrocytes coated with B active bacterial products are polyagglutinable. Absorption attempts to eliminate polyagglutinability will be reported. (Aided by Natl. Sci. Fndn.).

039. BLOOD–Leukocytes

1 *Physiology*

THE ROLE OF COMPLEMENT IN LEUKOCYTE-ERYTHROCYTE MIXED AGGLUTINATION. Parviz Lalezari* and Theodore H. Spaet. Dept. Hematol., Lab. Div., Montefiore Hospital, New York.

Leukocyte-erythrocyte mixed agglutination occurs when leukocytes of any type are mixed with erythrocytes and a hemolytic antibody active against the erythrocytes. This reaction has been found with ABO, hemolytic cold, and Donath-Landsteiner antibodies, but not with incomplete antibodies of the Rh type or those associated with acquired hemolytic anemia. As is the case with immune hemolysis, the mixed agglutination reaction is augmented by a thermolabile component in serum, and it is inhibited by cold incubation, EDTA, heparin, and hypertonic salt solutions. Both reactions have an optimal pH range, and are inhibited at extreme deviations from neutrality in either direction. The effective concentration of inhibitory reagents is proportional to the titer of hemolytic antibody; and if antibody titer is sufficiently high, no effective inhibition may be possible, with heparin or EDTA concentrations far in excess of those considered to be completely anticomplementary. Hemolysis is more susceptible to inhibition than mixed agglutination. The data show that complement enhances, but is not essential for mixed agglutination in the presence of adequate antibody. The reaction may be a tool for detection of subhemolytic antibody concentrations.

2 *Pathology*

SERUM ELASTASE INHIBITOR. Robert B. Schneider* and Roy L. Walford. U.C.L.A. School of Medicine, Los Angeles, Calif.

Serum elastase inhibitor is moderately elevated in some disease states and greatly elevated in pregnancy in some animals. In human pregnancy the elevation begins in the first trimester, increases to maximum in the third trimester and the immediate postpartum period, and returns to normal by six weeks postpartum. It is not elevated in the newborn. The inhibitor is also elevated in pregnant rats but not at any stage of pregnancy in the rabbit. ACTH and progesterone failed to produce elevation of the elastase inhibitor when injected into rats. The inhibitor activity is present in the alpha$_1$-globulin/albumin junction of electrophoretically fractionated serum. Inhibition is not related to the level of total serum proteins. It is important to determine inhibitor levels after one hour of incubation as substantially shorter periods yield non-reproducible results. (Supported by USPHS grant H-2538.)

3 *Pathology*

ANTI-GLOBULIN CONSUMPTION IN THE DETECTION OF LEUKOCYTE ANTIBODIES. Robert E. Anderson* and Roy L. Walford. U.C.L.A. School of Medicine, Los Angeles, Calif.

Separated human leukocytes were incubated with homologous putatively immune serum, thoroughly washed, and used to "adsorb" anti-human globulin serum. A significant decline in titer of the latter indicated a positive test, i.e., that the leukocytes had been "sensitized". A more quantitative method was to isolate anti-HG antibody, label with I^{131}, and directly measure adsorption of antibody by "sensitized" as compared to non-sensitized leukocytes. Non-specific adsorption of human globulin and of rabbit globulin by human leukocytes was also studied with labelling technics, with a view to developing optimum washing procedures. Normal human globulin was best washed off of leukocytes with Seligmann's solution, and rabbit globulin with 0.85% NaCl. Prior incubation with normal rabbit serum served partially to block non-specific adsorption of labelled anti-HG serum prepared in rabbits. With the above technics leukocyte antibodies were demonstrated in sera of patients with a variety of hematologic disorders and in patients who had received massive transfusion of living leukocytes at cardiac surgery. (Supported by USPHS grant A-1935.)

4 *Pathology*

THE INFLUENCE OF ANTICOAGULANTS ON THE LEUCOCYTIC STICKING REACTION WITHIN RABBIT EAR CHAMBERS INJURED BY HEAT, Fred Allison, Jr. and Margie G. Lancaster, (intr. by C. C. Randall) Univ. of Miss. Sch. of Med., Jackson, Miss. (Motion Picture)

Studies of rabbit ear chambers damaged by heat have suggested a direct relationship between the sticking of leucocytes to endothelium and the intravascular polymerization of fibrinogen to fibrin. In this study rabbits were given heparin sodium, sodium warfarin, or a combination of both for anticoagulation. Vascular dynamics and leucocytic behavior within undamaged ear chambers were not disturbed by these agents. Following injury by heat, the white cell sticking reaction developed as in controls even though anticoagulation as measured in vitro was maximal. Other aspects of acute inflammation both vascular and cellular were similarly unchanged by the anticoagulants. An unexpected finding was the enhanced formation of intravascular fibrin-like material in injured ear chambers of rabbits given heparin sodium. This was so even though clotting times were greater than 24 hrs.. Since at the conclusion of experiments formation of thrombi was not increased, it was thought heparin permitted a partial but reversible polymerization of fibrinogen. These data were construed as evidence against a coating of fibrin on cell surfaces as a primary cause of leucocytic sticking. (Supported by a grant from NIH).

5 *Pathology*

ISOLATION OF HIGHLY PURIFIED LEUKOCYTES FROM HUMAN BLOOD, A. R. Christlieb, A.J. Sbarra, W. A. Bardawil, St. Margaret's Hospital, Path. & Med. Res. Labs., & Tufts Univ. School of Med., Boston, Mass.

It is possible to isolate active, discreet WBC with an average purity of 99.9% and with an average recovery of 30%, by employing a Teflon homogenizer, from 50 to 500 cc. of heparinized human blood RBC are sedimented with 20% Dextran, centrifuged to recover WBC-RBC platelet mixtures, subjected to trauma* with a Teflon homogenizer to break up clumped cells, and sedimented to recover WBC."pure" cells were compared to control cells having a WBC: RBC ratio of 1:1 prepared by conventional methods. Respiratory studies were carried out and phagocytic activities were measured using killed bacteria. Results obtained with "pure" cells were comparable to those of control cells. Dye-reduction measurements compared well with those of Cooper et al, (J. Lab. & Cl. Med., 53, 457, 1959) using conventional cell preparations. With minimal RBC and platlet contamination, these cells should prove useful in enzymatic and immunological studies. Studies on the antigenic activity of these cells are in progress and detailed procedures & results will be described. (NIH Grants A2168 and B1962) *instantaneous

6 *Pathology*

LEUKOCYTE CHANGES IN NORMAL DOGS EXPOSED TO MICROWAVES. Roderick A.E. Thomson, Sol M. Michaelson and Joe W. Howland (intr. by I.L. Spar.). Univ. of Rochester School of Med. and Dentistry, Rochester, N.Y.

Exposure of normal dogs to 2800 Mc (pulsed), 165 mw/cm^2/120 mins. results in a 20.4% decrease in total WBC from initial value. Normal level is re-established one day after exposure. A 20.9% increase is evidenced immediately after 180 minutes exposure, with a further increase to 44.2% one day later. At 100 mw/cm^2/360 mins. no immediate change occurs, but one day later is increased 22.8%. Total WBC is increased 44.5% after 325 mins. at 200 Mc (continuous), 165 mw/cm^2. There is a decrease of 31.2% to 59.7% in lymphocytes immediately after all exposures. Immediately and 24 hrs. after all but the 120 min. exposure there is an increase in polys. A 67% cosinophil decrease occurs after exposures lasting 325 and 360 minutes. Latent cosinophil changes are variable. Results indicate specific leukocyte changes dependent on wavelength, power level, and exposure time occur in dogs exposed to microwaves. (Supported by a grant from USAF, Rome Air Development Center, Rome, N.Y.).

7 *Pathology*

STUDIES ON THE PATHOGENESIS OF FEVER. PRELIMINARY PURIFICATION OF LEUCOCYTIC PYROGEN. G.W.Rafter, R.D. Collins, and W.Barry Wood,Jr. (intr. by John L.Shapiro) Dept. of Microbiology, Johns Hopkins School of Medicine and Dept. of Biochemistry, Johns Hopkins School of Hygiene and Public Health, Baltimore, Md.

The febrile reaction is apparently due to stimulation of the thermoregulatory centers of the brain by one or more circulating endogenous pyrogens. There is abundant evidence that most, if not all, of these circulating pyrogens are released from damaged polymorphonuclear leucocytes. The purpose of this study was to investigate the chemical nature of leucocytic pyrogen. Leucocytic pyrogen was found to be inactivated by proteolysis, unaffected by butanol extraction, and soluble in 50% methanol. It is concluded that leucocytic pyrogen is a protein that is chemically distinct from bacterial endotoxins, from the heat-stable euglobulin "pyrexin" that Menkin has extracted from inflammatory exudates, and from the pyrogenic tissue polysaccharides of Landy and Shear. None of these latter pyrogens appears to be directly involved in the production of naturally occurring fevers. (Supported by a grant from the Life Insurance Medical Research Fund.)

040. BLOOD–Leukocytes–Leukemia

1 *Physiology*

AMINOPTERIN-RESISTANCE IN NON-LEUKEMIC RATS. P.G.Reizenstein (intr. by B.P.Cronkite) Brookhaven Nat. Lab.,Upton, N. Y. and Karolinska Hosp., Stockholm, Sweden.

If no resistance developed, chemotherapy would be satisfactory in many leukemias. Selection of resistant tumor cells is one mechanism for developing resistance, but little is known of part played by physiologic adaptation. 18/25 male rats receiving 0.025 mg Aminopterin (APGA) subcutaneously 3 times weekly had mean survival time of 31 (11-50) days. Blood and bone marrow changes are described (Reizenstein,P.: Bur.Soc.Hematol. 6, 253,1957). 7/25 "resistant" survivors lived 126 (110-134) days. "Resistant" survivors killed between 30 and 60 days had less marrow aplasia than random rats killed after 21 days. 7 separate 5-rat groups, pretreated with 0.025 mg APGA 3 times weekly for 30-60 days received, after a 2-week rest, a 10-fold dose. Mean survival (8.9-0.4 days) was significantly higher than in 6 simultaneous 4-animal groups of previously untreated rats (6.6±0.4 days). To determine if this relative resistance is due to selection of naturally resistant animals or physiologic adaptation, blood values were followed under continuous APGA treatment. Initially, RBC's fell at same rate in all animals but, after 2-3 weeks, increased in rats subsequently found resistant. Findings indicate that APGA-resistance occurs in non-leukemic rats, probably due to physiologic adaptation. This resistance is not stable; all animals finally die.

XUM

2 *Physiology*

A HYPOGLYCEMIC FACTOR IN LEUKEMIC TUMOR OF MICE. Murray N. Silverstein,* Khalil G. Wakim, Robert C. Bahn, Edwin D. Bayrd.* Mayo Clinic and Mayo Foundation, Rochester, Minnesota.

A fall in blood sugar was observed in AKR mice inoculated with leukemic tumor BW5147. Marked amelioration of diabetes resulted from the inoculation of alloxanized AKR mice with lymphatic leukemic tumor. The growth of this tumor was studied in normal and diabetic mice. The rate of tumor growth and the blood sugar values were correlated. Increase in tumor mass alone did not seem to explain the hypoglycemia seen in these mice. Consequently, three extracts of this tumor were made and tested for the presence of a hypoglycemic factor. Blood sugar values of diabetic mice were determined before and after injection of tumor extracts. A saline extract of the tumor showed no hypoglycemic effect; an extract made by the insulin-extraction procedure also had no hypoglycemic effect. An acetone extract of the tumor, however, exhibited a moderate hypoglycemic activity. Time-response curves demonstrated that the acetone-extractable material derived from 1 gm. of fresh tumor produced a fall in blood sugar of 7 per cent at 15 minutes, 30 per cent at 30 minutes, 25 per cent at 1 hour, and 7 per cent at 2 hours. Acetone extract of the lymphatic leukemia tumors removed from diabetic (alloxanized) tumor-bearing animals exhibited greater hypoglycemic activity than did similar extracts of tumors obtained from tumor-bearing animals that were not made diabetic.

3 *Biochemistry*

ALPHA-GLYCEROPHOSPHATE CYCLE AND LACTIC DEHYDROGENASE OF HEMATOPOIETIC CELLS IN NORMAL AND LEUKEMIC MICE. Bertram Sacktor and Arthur Dick*. Dir. Med. Res., Army Chem. Ctr., Md.

The alpha-glycerophosphate (a-GP) cycle, the oxidation of DPNH with concomitant reduction of dihydroxyacetone phosphate (DHAP) to a-GP coupled to the mitochondrial oxidation of a-GP back to DHAP, previously described from flight muscle and mammalian brain is especially active in normal mouse hematopoietic cells. Following implantations with four different types of experimental leukemia, a-GP dehydrogenase activities of bone marrow, isolated lymphocytes, lymph nodes and spleen are decreased to values as low as one-fifth normal. Lymphoid cells from solid tumors and from ascites fluid show even lower activities. In contrast, lactic dehydrogenase, which competes with a-GP dehydrogenase, increases in activity. As much as a three-fold increase is found in malignant bone marrow cells. The ratio of lactic to a-GP dehydrogenase activities, which is about 10 in normal lymphocytes, is increased many fold, reaching a value of 870 in solid tumors. The oxidation of a-GP by infiltrated spleen mitochondria is decreased to half normal whereas the oxidation of citrate cycle intermediates by these mitochondria is unchanged. Such findings suggest a metabolic disturbance in the a-GP cycle and suggest an inbalance in the hydrogen transfer mechanisms.

4 *Pathology*

BACTERIAL POLYSACCHARIDE AND CORTISOL IN ACUTE LEUKEMIA OF THE RAT. Claude R. Thomas and Russell S. Jones. Dept. of Pathology, Univ. of Utah Coll. Med., Salt Lake City, Utah.

Acute leukemia was produced by intravenous injection of cells from the Murphy-Sturm lymphosarcoma in young male Holtzman rats. Immediately prior to the rapid and marked increase of lymphoblasts in the peripheral blood but when the leukemic cells were numerous in the bone marrow and viscera, the growth of the young rats ceased. When polysaccharide from Klebsiella pneumoniae was injected intravenously .1 mg/100 g body weight in the aleukemic phase the leukemic cells disappeared from the marrow and viscera, but when the bacterial product was injected after the onset of marked elevation of primitive cells in the peripheral blood few cures resulted. Rats cured of the leukemia were resistant to either the leukemia or lymphosarcoma produced by the Murphy-Sturm tumor cells. Daily subcutaneous injections of cortisol shortened the leukemic phase of the rapidly fatal disease and prevented the anti-leukemic action of the bacterial polysaccharide. (Supported by a grant from Nat. Cancer Inst.)

5 *Pathology*

EFFECTS OF AMINO ACID OVERLOADS IN MYELOCYTIC LEUKEMIA. W. J. Harrington, G.K. Arimura*, K. Muench*, and D. Harkness*. Dept. of Med., Washington Univ. Sch. of Med., St. Louis, Mo.

Single alpha amino acids were given I.V. in a dose of 20 gm./day x2. No in vivo effects were noted in normal subjects or in patients with leukemoid reactions or acute myelocytic leukemia (whether occurring de novo or as an end stage of chronic leukemia). However, in 91 trials on patients with chronic myelocytic leukemia (CML) certain patterns of response to the sulfur containing amino acids were noted: 1) a prompt increase in circulating white blood cell count (WBC), maximal in 1-2 days post-transfusion with restoration to control values after 3-4 days, 2) no change in differential counts or other blood or bone marrow cell values, 3) an increase in serum B_{12} levels paralleling the changes in WBC, 4) an increase in uric acid excretion. L-methionine induced a greater increase in WBC (32.2%) than L-cysteine (16%). D-methionine was inactive as were 15 other amino acids. Baseline serum levels of L-methionine were normal in all subjects and less than 5% of the administered sulfur was recovered in the urine over a 2 day period post-infusion. In vitro, L-methionine failed to stimulate the uptake of C_{14} formate, adenine, or orotic acid into DNA and RNA but suggestive in vivo enhancement was noted. (Supported by grant P-143B from the American Cancer Society).

6 *Pathology*

THE PHOSPHOLIPIDS IN HUMAN LEUKEMIC CELLS. Barry G. Firkin and William J. Williams (intr. by William J. Harrington). Dept. Med., Washington Univ. Sch. of Med., St. Louis, Mo.

During an investigation of lipid metabolism in leukemia, the phospholipid components of human leukemic cells were isolated and identified. Chloroform-methanol extracts of white cells taken from patients with acute leukemia, chronic myelocytic and chronic lymphocytic leukemia were analyzed by silicic acid column chromatography. Three major peaks were obtained. Appropriate paper chromatographic techniques both before and after acid hydrolysis of the fractions showed the first peak to contain phosphatidyl ethanolamine and phosphatidyl serine, the second phosphatidyl choline and inositol and the third sphingomyelin. No major differences were apparent in the types of leukemic cells investigated. Platelet and red cell extracts prepared in a similar manner revealed the same components although the red cell extracts contained less inositol than those of the platelet or white cell. P^{32} was administered to patients with chronic myelocytic leukemia and its incorporation into the phospholipids of red cells, white cells and platelets was followed by autoradiography of the samples taken at suitable time intervals. Incorporation of the isotope was readily demonstrated after 18 hours, and by 120 hours all components were strongly labelled but for phosphatidyl serine. (Supported by grant P-143B from the American Cancer Society.)

7 *Pathology*

HETEROTRANSPLANTATION OF SPONTANEOUS AKR-LEUKEMIA INTO NEWBORN RATS. M.H. Kirsten (intr. by H. Lisco) Dept. of Pathology, University of Chicago, Chicago, Ill.

Successful heterologous transplantation of malignant tumors of human or animal origin rests largely on the absence of an effective immunological response in the recipient animal. Lymphomas and leukemias are particularly rigid in their host requirements. The phenomenon of acquired immunological tolerance has first been utilized for homologous and heterologous tissue grafting by Koprowski (Fed. Proc. 16, 592, 1957).

The present experiments were undertaken to adapt this method to the transplantation of spontaneous AKR-leukemia into rats. However, instead of inoculating rats in utero the recipients received the inoculum subcutaneously, intraperitoneally or intravenously within 12 hours post partum. Cell suspensions of spleen or a combination of spleen, thymus and lymph nodes resulted in tumor growth in a considerable number of the newborns receiving intravenous or intraperitoneal injections. Re-inoculation of the same donor-leukemia 6-8 weeks after birth increases the number of takes. Factors influencing the acceptance of heterologous grafts and the results of serial and back transplantations will be discussed. (Supported by a grant from U.S.P.H.S. #C-4311.)

8 *Pathology*

EFFECTS OF X-RAYS ON NORMAL AND LEUKEMIC HUMAN LYMPHOCYTES (motion picture). Robert Schrek. Veterans Admin. Hosp., Hines, Ill.

Time-lapse cinemicrographic studies showed that x-irradiation (1000 r) of normal human lymphocytes produced intranuclear vacuolization and death of 90% of cells in 1.5 days. Non-irradiated lymphocytes underwent the same cytologic changes but at a slower rate with 90% of cells dead in 9.0 days. A perceptible decrease in the survival time of human lymphocytes was observed after irradiation with 5 r. Irradiation with 4000 r killed normal lymphocytes in a few hours by "delayed fixation" followed by shrinkage and autolysis of the nuclei. Leukemic lymphocytes from patients were either round and mature or immature with flagella-like processes. The leukemic lymphocytes from most patients were as sensitive to x-rays as normal cells and died by intranuclear vacuolization. About 20% of the patients had leukemic blood lymphocytes resistant to irradiation. Radiosensitive and radioresistant lymphocytes had the same cytologic appearance. Fifteen patients with radioresistant lymphocytes responded poorly to therapy and had a median survival time of 4 months as contrasted to 19+ months for 36 patients with sensitive lymphocytes.

041. BLOOD–Proteins

1 *Physiology*

ALTERATIONS OF ELECTROPHORETIC MOBILITIES OF SERUM PROTEINS AND LIPOPROTEINS BY IODINIZED CONTRAST MEDIA. H. Kutt and F. McDowell, (intr. by L.F. Chapman) 2nd (Cornell) Neurological Service, Bellevue Hospital, N.Y.C.

Mixing of human serum with iodopyracet or sodium or methylglucamine ditrizoate in ratios 1:1 to 10:1 caused marked alteration of the mobilities of serum proteins and lipoproteins in paper, starch granule and starch gel electrophoresis. Albumin and alpha and beta globulin peaks flattened or disappeared depending upon the amount of contrast media in the mixtures, whereas an unusually high peak appeared in the gamma globulin region. Some protein, however, migrated towards the anode faster than the normal albumin front. This area corresponded with the peak of the contrast medium, indicating that some protein was attached to the latter. The alpha and beta lipoprotein peaks disappeared and a high lipid containing peak appeared in the gamma region. Similar changes in the protein and lipoprotein patterns were observed in the blood that was drawn from the vein of a patient who was receiving contrast medium for intravenous urography. The magnitude of the protein and lipoprotein changes varied considerably among individual patients. It was greatest in patients with pre-existing abnormal protein patterns such as multiple myeloma and macroglobulinemias.

2 *Physiology*

PAPER ELECTROPHORETIC ANALYSES OF SERA OF HEALTHY NEGRO COLLEGE MEN ENGAGED IN SPORT COMPETITION. T.F. Johnson*, and Harry Y.C. Wong, Dept. of Physiology, Howard Univ. School of Med., Washington, D.C.

Physical activity is beleived to cause increase in concentration of serum proteins. Data obtained on college swimmers during a five month training-competitive period when compared with subsequent non-competitive interval showed no significant differences in protein components. However, data relative to the opening meet of the season did show significant increase in albumin and a complimentary change in beta globulin fraction between pre-meet and next morning values. Mean values for albumin and globulin fractions prior to beginning the training-conditioning regimen, compare favorably with paper electrophoretic serum protein values appearing in the literature for normal healthy subjects. Values obtained prior to exercise, during the competitive period or during the subsequent non-competitive period do not support the opinion that gamma globulin is higher in adult Negroes than in White persons. "Normals" for albumin and all globulin fractions were close to mean values derived from a compilation of papers on electrophoresis for normal White subjects. Supported by grants from PHS grant H-2420, (C3), American and Washington Heart Associations.

3 *Biochemistry*

HUMAN AND BOVINE FIBRINOGENS: CHROMATOGRAPHIC, ULTRACENTRIFUGAL AND IMMUNOLOGIC CHARACTERIZATION. Morton D. Prager, R. J. Speer and Phil S. Work (intr. by Morton Mason). Wadley Res. Inst. and Blood Center and Grad. Res. Inst. of Baylor Univ.

Bovine and human fibrinogens were chromatographed on DEAE-cellulose using the glycine-phosphate buffer system previously described (J.Lab.Clin.Med.54:685,694;1959). Complex patterns were obtained with coagulable protein appearing in 3-5 peaks. After concentration these chromatographic fractions gave ultracentrifuge patterns with up to 3 sedimenting boundaries. It appears that this heterogeneity resulted from interaction of fibrinogen with protein contaminants of Fraction I. When Fraction I, purified by the procedure of Blomback (Arkiv Kemi 10:415;1956), was chromatographed in the presence of 0.1M urea to prevent coagulation, a single broad peak emerged, and protein from several portions of the chromatogram was > 95% coagulable. The column charges and fractions were homogeneous ultracentrifugally, giving added weight to the idea that the heterogeneity observed with crude Fraction I was not created by the chromatographic process. Rabbit antisera to human and bovine Blomback preparations were used to compare column charges and fractions by means of the Ouchterlony technique. The bovine fibrinogen charge yielded 2 lines, the major of which gave a reaction of identity with the fractions of the chromatographic peak. (Supported by an NIH grant.)

4 *Biochemistry*

IODINATION OF STREPTOKINASE (SK). E. C. De Renzo and P. K. Siiteri*. Biochemistry Research Dept., Biochemical Research Section, Lederle Labs., American Cyanamid Co., Pearl River, N.Y.

Iodination of SK was attempted by layering solutions of SK buffered at pH 8.5 over equal volumes of CCl_4 solutions containing I_2^{127} and I_2^{131} and gently agitating the lower phase. Reaction was allowed to proceed for varying periods of time at 5°C. At the end of a selected time the reaction products in the aqueous layer were precipitated by the addition of 10% NaCl at pH 3.0. The precipitate was redissolved at pH 8.0 and exhaustively dialyzed to remove unbound iodine. Chromatography of the reaction products was then carried out on DEAE-cellulose. Protein, radioactivity and SK activity were determined on effluent fractions. Depending upon the length of reaction time products were obtained which were calculated to contain ratios of atoms I/mole of SK which varied from 1/1 to 28/1. In no instance did the main radioactivity peak coincide with the SK activity peak. Furthermore, except for the case where 28 atoms of I were introduced per mole of SK the main radioactivity peak was associated with a minor protein peak. The results obtained by this procedure therefore do not provide evidence for the formation of a biochemically active molecular species of iodo-SK.

5 *Biochemistry*

A PROTEIN MOIETY INHIBITORY TO HeLa CELL GROWTH IN SERA OF BURNED CHILDREN. R.I. Lytle, L.F. Miller and S.R. Rosenthal (intr. by E.R. Kirch.) Naval Med. Research Unit #4, Great Lakes, Ill., and Univ. of Ill. College of Med., Chicago.

Sera from acutely burned children were studied electrophoretically with a modified Aaransson technique to search for abnormal moieties not resolved by conventional methods. An abnormal component migrating more rapidly than the albumin fraction was observed when the sodium salt of ethylene-diamine tetra-acetic acid was substituted in Aaransson's buffer system at a pH of 9.1. A reduction in alpha-2 roughly equivalent to the abnormal component was noted when patterns obtained by the conventional barbital and modified techniques were compared suggesting further resolution of the alpha components with the latter. The abnormal moiety was associated with inhibition of HeLa cell growth in 17 specimens from 10 burned patients with levels of 3 to 12.5% and with absence of inhibition in 3 specimens from 3 patients with levels of 3 to 5%. The moiety was not detected in serum from unburned children. (Aided by a grant from the Office of Naval Research, NR 114 161.) (The opinions and assertions contained herein are those of the authors and are not to be construed as official or reflecting the views of the Navy Department or the Naval Service at large.)

042. BLOOD–Proteins–Albumins

6 *Biochemistry*

THE ANTIOXIDANT ACTIVITY OF VERTEBRATE BLOOD. Albert A. Barber (intr. by W.F.H.M. Mommaerts.) Zoology Dept., UCLA

The formation of lipid peroxides in aerobically incubated rat brain homogenates is inhibited by the addition of antioxidants from various animal tissue extracts. Vertebrate blood was examined for this activity. The antioxidant activity of rat, turtle, and frog sera was found to be non-dialyzable, heat labile, and reversible by small amounts of iron. Separation of the serum proteins by continuous flow electrophoresis resulted in a partial purification of the antioxidants. In rat serum, one component possessed all of the activity found in whole serum. The protein content of the purified preparation required for 50% inhibition of peroxide formation was 0.1 mg as compared to 1.0 mg required by the whole serum, a tenfold purification. Iron reversibility studies indicate that the antioxidant of rat serum is associated with the iron binding protein, β-globulin. Electrophoresis of turtle blood resulted in the separation of two components possessing antioxidant activity with the slow moving component being approximately three times more active than the fast one. The antioxidant activity of both components is reversible by iron. Frog serum also contains at least two, and possibly three components possessing antioxidant activity. The active components in both turtle and frog serum have not as yet been identified. (Supported by a grant from the U.S. Atomic Energy Commission.)

7 *Nutrition*

A NEW SERUM PROTEIN IN PREGNANT RATS. George H. Beaton and Anne Selby (intr. by E. W. McHenry) Department of Nutrition, School of Hygiene, University of Toronto, Toronto, Ontario, Canada.

Protein metabolism is known to undergo alteration during pregnancy. Using the starch gel electrophoresis method of Smithies, it is possible to demonstrate a distinct new band in the sera of pregnant rats. Other workers have show a distinct band in the sera of pregnant women. In both cases, the new band appears at about mid-pregnancy. The nature of the rat protein has been studied using two dimensional filter paper - starch gel electrophoresis. Similar bands have been seen in the sera of very young rats, and in the sera of rats bearing the Novikoff hepatoma. This band is not seen in the sera of normal nonpregnant adult rats.

8 *Immunology*

SERUM SPECIFICITIES IN THE ANTHROPOIDEA. Morris Goodman and M. D. Poulik.* Wayne State Univ. & Children's Hospital, Detroit, Michigan.

In order to view the serum specificities of man in broader biological perspective, the sera of some of the Anthropoidea were compared by two dimensional starch gel electrophoretic and immunological techniques. Species specific starch gel patterns (of from 16 to 25 components) reflected taxonomic relationships between the macaque monkeys (rhesus and cynomolgus), chimpanzees, and human beings. The rhesus and cynomolgus monkeys showed the most similarity in their patterns, yet were separated by the much greater quantity of cynomolgus gamma$_1$ globulin. The intraspecific variation, most pronounced for man, never impinged upon the interspecific variation of patterns. To facilitate comparisons, we identified immunologically in the starch gels the counterparts of known human proteins. Certain ones, such as transferrin and alpha macroglobulin, were positioned differently in the chimpanzees than in the humans and macaques; others, such as post and prealbumins, set the macaques apart. The significance of the results as they relate to the detection of intraspecific differences in proteins between human beings will be discussed. (Grant support was from the National Institutes of Health, National Multiple Sclerosis Society, and Children's Fund of Michigan.)

1 *Physiology*

FURTHER STUDIES ON THE PREPARATION AND BEHAVIOUR IN MAN OF I^{131}-LABELLED HUMAN SERUM ALBUMIN. Y. TAKEDA* AND E. B. REEVE, DEPT. OF MEDICINE, UNIV. OF COLORADO SCHOOL OF MEDICINE, DENVER.

Last year (Fed. Proc. 18, 155; 1959), we and Franks reported studies of the behaviour in man of fairly pure human serum albumin labelled with I^{131}. Subsequent study, by paper and starch gel electrophoresis and DEAE cellulose chromatography, showed these albumin preparations to contain up to 5 per cent of alpha globulins, a portion of which had a higher specific activity than that of the albumin. Since the presence of this amount of labelled globulins makes the measurement of the catabolic rate of I^{131}-albumin a little uncertain, the original albumin, prepared by salt fractionnation, was further purified on DEAE cellulose columns, with the removal of much of the residual alpha globulins. Studies of the improved preparation when injected into 4 volunteers--also the donors of the serum albumin--yielded half-lives ranging between 18 and 23 days (mean 20.8) and an average catabolic rate of 0.140 g albumin per kg body weight per day. The differences in half-life and catabolic rate between the original and the new preparations are not statistically significant.

2 *Biochemistry*

ELECTROPHORETIC BEHAVIOR OF HUMAN SERUM ALBUMIN AT ACID pH. Joseph Harris and Suzanna Goodyear (intr. by John A. Muntz) Dept. of Biochemistry, Albany Medical College, Albany, New York.

A microelectrophoretic study was made of the variations produced in the patterns of purified human serum albumin in succinate and benzoate buffers, pH 3.5 to 4.5, with and without phosphomolybdate (PMo). In succinate, u = 0.10, single boundaries were observed below pH 3.5 and above pH 4.3. Within these values, heterogeneity was obtained. Three major boundaries were clearly seen at pH 3.84 and 3.73. Addition of 0.88 uM PMo decreased the mobility and resolution of the boundaries. At 2.0 uM PMo two well-resolved boundaries were observed; the more mobile may be a PMo complex. Succinate, u = 0.01, gave complex boundaries, as did benzoate, u = 0.01. In benzoate at pH 4.48, the migration of the single boundary was reversed. The boundaries of defatted albumin in succinate, compared to those of regular albumin, were more mobile and apparent homogeneity occurred at a higher pH, i.e., 3.64. (Supported by PHS grant No. 1768-C2.)

3 *Biochemistry*

OPTICAL ROTATORY DISPERSION STUDIES OF ALBUMIN-VASOFLAVINE COMPLEXES. Joseph J. Betheil and Paul M. Gallop (intr. by Sasha Englard). Dept. of Biochemistry, Albert Einstein Coll. of Med., Yeshiva University, New York.

Vasoflavine, a methylated, sulfonated, benzthiazole derivative, interacts with serum albumins with increased fluorescence of the mixture. When studied spectrofluorometrically species differences are observed in the excitation and emission maxima with regard to both wavelength and emission intensity. The optical rotatory dispersion of the dye-albumin complexes has been studied and compared with that of the albumin alone for both human and bovine serum albumin. It has been observed that dye-albumin complexes show anomalous optical rotatory behavior. Vasoflavine alone has no optical activity. When bovine serum albumin is guanidinated, anomalous behavior is not observed in the presence of Vasoflavine. Deaminated bovine serum albumin however exhibits anomalous dispersion in the presence of the dye. It is believed that the anomalous behavior observed is due to rotary dichroism which results from interaction of the dye with specific asymmetric sites on the albumin molecule. Due to the chromophoric nature of the group which interacts, anomalous rotatory behavior would be expected due to the Cotton effect. Blout and Stryer (Proc. Natl. Acad. Sci., 45, 1591 (1959) studying poly-α-L-glutamic acid have come to the same conclusion regarding the dye-peptide interaction.

4 *Biochemistry*

NON-CIRCULATING EXTRAVASCULAR SERUM ALBUMIN. William R. Jevell, Jose C. Gan, and Henry Jeffay, (intr. by Bernard Weissmann). Univ. of Ill., Coll. of Med., Chicago, Ill.

Although many investigators accept the existence of a pool of non-circulating extravascular serum albumin, the anatomical location of this pool has not been reported. The total (circulating and non-circulating) serum albumin content of an organ can be determined immunochemically, if it is assumed that the vascular and extravascular proteins are immunologically the same. The circulating albumin can be determined by measuring the radioactivity found in the organ 2-5 minutes after the injection of pure albumin-S-35 of known specific activity, if it is assumed that none of the labeled albumin leaves the vascular compartment in this short time interval. The amount of non-circulating albumin is the difference between the total and the circulating albumin. All 10 tissues studied contained appreciable quantities of extravascular albumin. Most tissues had approximately the same albumin content per g. of tissue, but the heart, spleen, and kidney had much larger amounts.

2 *Immunology*

THE ROLE OF Ca^{++} AND Mg^{++} IN COMPLEMENT FIXATION TESTS. E. H. Fife, Jr. (intr. by E. L. Becker). Dept. of Serology, Div. of Communicable Dis., Walter Reed Army Inst. of Research, Wash., DC.

Although the role of Ca^{++} and Mg^{++} in immune hemolysis has been extensively studied, diverse opinions have been expressed concerning their use in complement fixation tests (Browne et al, Am. J. Clin. Path. 24:934, 1954; Kolmer et al, ibid. 24: 946, 1954). It is well known that in immune hemolysis Ca^{++} and Mg^{++} each functions in a specific manner, Ca^{++} protecting against complement (C') decay and Mg^{++} enhancing the hemolytic properties of the reagent. However, the role of these divalent cations in complement fixation reactions is not as well understood. The present quantitative studies indicated that Ca^{++} and Mg^{++} concentrations giving maximum hemolytic activity and stability of C' also elicited maximum fixation of C' by antigen-antibody complex. On the other hand, a relative excess of either cation inhibited the specific action of the other. Thus, addition of Ca^{++} alone significantly increased stability but markedly inhibited hemolytic activity of C'. Conversely, addition of Mg^{++} alone effectively increased hemolytic activity of C' but also greatly increased the lability of the reagent. The findings indicate that complement fixation procedures may be improved by incorporating proper concentrations of Ca^{++} and Mg^{++} in test reagents, whereas addition of either cation alone seems contraindicated.

5 *Pathology*

IN VIVO MEASUREMENT OF THE DEGRADATION RATE OF NORMAL ALBUMIN BY THE WHOLE-BODY GAMMA SPECTROMETER. Stuart W. Lippincott, Stanton H. Cohn and James S. Robertson. Med. Dept., Brookhaven Natl. Lab., Upton, N. Y.

Customarily the degradation rate of I^{131} labeled normal albumin in man has been determined from plasma or serum concentration curves and/or from urinary excretion data. The former method has been compared in two patients (with different diseases) with a new method employing the *in vivo* whole-body counting technique which permits measurement of very low levels of an internally deposited gamma emitter. In the first patient the half-lives of the I^{131} labeled albumin were 19 and 19.5 days respectively and in the second patient, 15 and 15.4 days. This new procedure makes possible the clinical investigation of turnover of albumin as well as other proteins with the administration of as little as one or two microcuries of the radioisotope used. The inconvenience to the patient of multiple blood sampling and to the investigator of tedious radiochemical analyses of excreta are eliminated. (This research was supported by the U. S. Atomic Energy Commission)

3 *Immunology*

KINETIC STUDIES OF THE REACTION BETWEEN $EAC'_{1,4}$ AND PURIFIED SECOND COMPONENT OF GUINEA PIG COMPLEMENT (C'_2).

Tibor Borsos and Herbert J. Rapp. Dept. Microbiology, Johns Hopkins School of Med., Baltimore, Md.

Formation of $EAC'_{1,4,2}$ from $EAC'_{1,4}$ by action of purified C'_2 proceeds without lag, indicating that reaction of one site (S) on the cell in the condition $SAC'_{1,4}$ with C'_2 to form $SAC'_{1,4,2}$ is necessary and sufficient to convert a cell to $EAC'_{1,4,2}$. On addition of C'_2 to $EAC'_{1,4}$, the number of $SAC'_{1,4,2}$ on the cell increases with time to a maximum which depends on the amount of C'_2 and then declines due to decay to $SAC'_{1,4}$. The maximal level of $SAC'_{1,4,2}$ varies linearly with the quantity of C'_2. The time needed to reach the maximum depends only on the concentration of $SAC'_{1,4}$ in the mixture. A mathematical theory has been derived and the results described above conform with this theory. This theoretical treatment makes it possible to measure C'_2 on a molecular basis. Guinea pig serum contains about 5×10^{13} molecules of C'_2/ml, assuming no unfruitful reactions resulting in loss of C'_2.

043. BLOOD–Proteins–Complement

1 *Biochemistry*

BINDING OF RADIOACTIVE COMPLEMENT COMPONENTS. F. Haurowitz and J. D. Hawkins*, Dept. of Chem., Indiana Univ., Bloomington, Ind.

In order to decide which of the four components of complement (C') are bound to antigen-antibody precipitates (AgAb) or to sensitized red cells, we prepared ^{35}S- or ^{14}C-labeled C' fractions from the sera of guinea pigs injected with labeled amino acids. The same results were obtained with ^{35}S and with ^{14}C. Significant amounts of radioactive C'_1, the first component of C', were bound by AgAb. When AgAb precipitates after treatment with nonradioactive C'_1, C'_{1+4}, or C'_{1+4+2} were exposed to radioactive C'_4, C'_2 or C'_3, respectively, no further radioactivity was taken up. Sensitized erythrocytes (EA) bind radioactive C'_1 which remains bound to the stroma after hemolysis. The EAC'_1 complex does not bind radioactive C'_4. We have not yet been able to decide whether radioactive C'_2 is bound to EAC'_{1+4}. We find however uptake of radioactive C'_3 by EAC'_{1+4+2}. Our results indicate that some of the components of C' exert their action without being irreversibly bound to AgAb or EA. They may act as enzymes or may be bound only temporarily and then released or degraded by the action of adsorbed C'_1 or of other enzymes. (Supported by grants of the U.S.Publ.Heath Service and the Natl.Science Foundation, and by contracts of Indiana Univ. with the Office of Naval Research and the Atomic Energy Commission).

4 *Immunology*

EFFICIENCY OF COMPLEMENT (C') AT DIFFERENT CONCENTRATIONS OF SENSITIZED ERYTHROCYTES (EA). D. L. McVickar (Intr. by C. F. Pait.) Dept. of Infectious Diseases, U.C.L.A. Medical Center, Los Angeles, California.

C' titrations at different EA concentrations are known to yield a family of regression curves with a common focus, when log C' is plotted vs. probit % hemolysis. Simultaneous C' titrations were performed with 12 different EA concentrations ranging from 10^7 to 2×10^9 EA/ml. The interrelationships between EA concentrations and the values determined for $RD_{50}C'$ and σ were found amenable to precise mathematical formulation, and could be expressed by the following two equations:

(1) $EA = a(RD_{50}C' - b)^c$, and (2) $\sigma = d + e \log RD_{50}C'$.

From equation (1) it is apparent that a threshold ("non-effective") amount of C' (parameter b) must be subtracted from the found value of $RD_{50}C'$ in order to obtain the effective $RD_{50}C'$. A linear relationship can then be shown between the log of the effective amount of C' per EA required to lyse 50% EA, and the log of the EA concentration. Thus it may be concluded that although the efficiency of C' increases as EA concentration increases, the rate of change in C' efficiency remains constant over the entire range of EA concentrations.

XUM

5 *Immunology*

A PRECIPITIN FOR AGGREGATED HUMAN FRACTION II, PRESENT IN NORMAL HUMAN SERA, AND WITH PROPERTIES LIKE THE FIRST COMPONENT OF COMPLEMENT. Angelo Taranta and Howard S. Weiss (Int. by H. F. Wood), Irvington House and Dept. of Medicine, N.Y.U. College of Medicine, New York.

Normal human sera (NHS), mixed with preparations of aggregated human serum fraction II (AHFII) were found to yield a precipitate. Quantitative determinations of the precipitates obtained using equal amounts of NHS and increasing quantities of AHFII, revealed three zones, characterized by increasing, maximal, and decreasing precipitates. Previous exposure of NHS to unrelated antigen-antibody complexes inhibited the ability of the serum to precipitate with AHFII, pointing to a similarity between serum complement and this normal precipitating factor. Fractional inactivation of complement components revealed that: (1) Heat-inactivated serum, and the supernatant of NHS dialysed against dilute acid buffers do not precipitate with AHFII. (2) The redissolved precipitate of NHS dialysed against dilute acid buffers, the zymosan-inactivated serum and the hydrazine-inactivated serum still precipitate with AHFII, although the recovery of precipitating activity is not complete. These data indicate that normal human serum contains at least one factor which precipitates with AHFII. This factor behaves like the first component of complement in a number of significant ways, and might prove to be identical with it.

6 *Immunology*

INHIBITION OF HUMAN C'1-ESTERASE BY ITS PARTIALLY PURIFIED HUMAN SERUM INHIBITOR. I.H. Lepow. Institute of Pathology, Western Reserve Univ., Cleveland.

The esterolytic and C'4-inactivating properties of human C'1-esterase are inhibited by a heat-labile globulin which has been partially purified from human serum (Fed. Proc. 18:591, 1959). In the present study, the kinetics of interaction of partially purified C'1-esterase with its partially purified serum inhibitor was investigated. Inhibition was measured by the extent of inactivation of C'4 at 2° by mixtures of C'1-esterase and serum inhibitor previously reacted under controlled conditions. The source of C'4 was serum heated at 56° for 30 min. (C'1-and inhibitor-deficient). Similar experiments were performed with C'1-esterase adsorbed to specific precipitates of pneumococcus S-III-rabbit anti-S-III. In both cases, inhibition was time-dependent, the rate increasing with temp. between 0° and 37°. The rate was also markedly dependent on initial conc. of C'1-esterase or inhibitor but was relatively insensitive to variation of pH, ionic strength or cation conc. The data will be discussed in relation to competition between C'4 and inhibitor for enzyme sites on C'1-esterase and the functional role of C'1-esterase in complement-fixation.

7 *Immunology*

FURTHER PURIFICATION OF A HUMAN SERUM INHIBITOR OF C'1-ESTERASE. J. Pensky (Intr. by I. H. Lepow) Institute of Pathology, Western Reserve Univ., Cleveland.

To elucidate the nature of a human serum globulin (EI) which inhibits an esterase derived from partially purified human C'1, further attempts were made to purify EI by column chromatography. EI was measured by its ability to inhibit stoichiometrically the esterolysis of N-acetyl-L-tyrosine ethyl ester under standard conditions (Levy and Lepow, Proc. Soc. Exp. Biol. Med. 101:608, 1959). Material obtained by Dowex-2 chromatography of 40 percent ammonium sulfate supernatant fraction of serum (Pensky and Levy, Fed. Proc. 18:591, 1959) was pooled, concentrated by lyophilization and re-chromatographed on diethylaminoethyl cellulose columns at various conditions of pH and ionic strength gradient. At pH 6.0 the inhibitor emerged in a single peak in the ionic strength gradient between 0.06 and 0.31. The best fractions were purified approximately 500-fold with respect to serum. Data relating to further purification and some physical and chemical properties of EI will be presented. (Supported by Public Health Service Grant E-1255 (C3)).

8 *Immunology*

INITIATION OF DONATH-LANDSTEINER HEMOLYSIS BY C'1-ESTERASE AND INHIBITION BY INHIBITOR OF C'1-ESTERASE. Carl F. Hinz, Jr.,* Mary E. Picken,* and Irwin H. Lepow. Western Reserve Univ., Cleveland, Ohio.

The hemolytic effect of human complement in the Donath-Landsteiner reaction, an immune human hemolytic system requiring sequential cold and warm reactions, is initiated by the first component (C'1) in the initial cold reaction, and is completed by the remaining components in the subsequent warm reaction. The partially purified enzymatically active material from C'1, (C'1-esterase), can be substituted for whole serum in the initial cold reaction, suggesting that esterase properties may be responsible for initiation of hemolysis. Supporting evidence is: a) Partially purified human serum inhibitor of C'1-esterase blocks stoichiometrically the reaction between C'1-esterase and erythrocytes in the initial reaction. At high concentrations of C'1-esterase and inhibitor blocking occurs instantaneously at 1°. b) The initial effect on the cell of C'1-esterase or whole serum can be overcome by subsequent incubation with inhibitor. This reaction is time dependent; the rate increases from 1 to 37°, and depends on initial concentration of inhibitor.

9 *Immunology*

ISOLATION OF A HEAT LABILE SERUM COMPONENT WHICH INTERACTS WITH γ-GLOBULIN AGGREGATES. H.J. Müller-Eberhard* and H.G. Kunkel, Rockefeller Institute, New York.

Addition of soluble γ-globulin aggregates to various normal human sera followed by incubation at 4°C leads to the formation of a precipitate. Dissolved in glycine buffer pH 3 it consisted mainly of two ultracentrifugal components: the rapidly sedimenting aggregates and a protein with an $S^{\circ}_{20,w}$=10.7S. The latter constituted 10-15% of the precipitated material. In addition, small amounts of a 7S and 0-2% of a 19S component were observed. Aggregated γ-globulin induced precipitates were also obtained from the euglobulins of fresh serum and from serum lacking C'_2, C'_3, C'_4. They could not be obtained from serum heated at 56°C for 30 min., serum lacking C'_1 or the serum pseudoglobulins. Precipitates from various sources resembled each other in ultracentrifugal composition, especially with respect to the invariably present 10.7S component. This component was isolated by extracting washed precipitates with phosphate buffer pH 5.3, ionic strength 0.3, followed by preparative ultracentrifugation of the extract. A highly purified preparation of the 10.7S component retained the capacity to precipitate soluble γ-globulin aggregates. The 19S component seen in some precipitates appears with respect to s-rate and affinity to aggregates to be related to rheumatoid factor whereas the 10.7S component in view of its heat lability, euglobulin nature, absence from R_1 and ability to interact with γ-globulin aggregates resembles C'_1.

10 *Immunology*

APPROACH TO THE PROBLEM OF IDENTIFYING A COMPLEMENT SUBSTRATE OR RECEPTOR IN CELL WALLS. A.C. Wardlaw. (intr. by B. Cinader) Connaught Medical Research Laboratories, Toronto, Ontario.

Erythrocytes and Gram negative bacteria in general are sensitive to complement whereas Gram positive bacteria are not. The former may therefore have complement substrates or receptors in their cell walls which are absent from the latter. The substrate may be lipid and/or protein, major components of erythrocyte and Gram negative, but not Gram positive, cell walls. Those particular Gram negatives which are highly sensitive to complement differ, it is suggested, from less sensitive ones in having a larger proportion of their wall occupied by complement substrate sites, and a smaller proportion occupied by lipopolysaccharide. In support of these ideas, it will be shown that the cell wall of a highly complement-sensitive strain of Escherichia coli is composed mainly of protein (approx. 65%) and phospholipid (approx. 25%) with much smaller amounts of lipopolysaccharide (3%) glucosamine and muramic acid (3%) and RNA (1%). The optional conditions for lysis of whole cells by normal human serum are pH 8.3 (broad optimum), ionic strength 0.06 (sharp optimum), veronal buffer and 37°. Walls prepared by Mickle disintegration showed, after incubation with normal human serum, 25% reduction in turbidity, 15% loss in weight, 50% loss of phospholipid and an apparent loss of bacterial wall protein and gain of serum protein.

044. BLOOD–Proteins–Globulins

1 *Biochemistry*

CORRELATION BETWEEN SERUM Cu, CERULOPLASMIN ACTIVITY AND C-REACTIVE PROTEIN (CRP). E. W. Rice (intr. by C. J. Cox.) Presbyterian Hosp., Pittsburgh, Pa.

CRP and hypercupremia occur together in many inflammatory conditions. In human plasma about 90% of the Cu exists as the oxidase "ceruloplasmin". Despite this observation, many methods for serum oxidase (ceruloplasmin) activity have not demonstrated a satisfactory linear relation between oxidase activity and Cu concentration. It was with a view of establishing a more precise relationship among the 3 afore-mentioned parameters of inflammation that the present study was undertaken. 205 sera were analyzed for Cu (Rice, E. W., J. Lab. & Clin. Med., in press), oxidase activity (Houchin, O.B., Clin. Chem., 4, 519 (1958), modified), and CRP; 70 additional samples were analyzed for Cu and CRP only, with results summarized as follows: (1) There was a very strong positive linear correlation between serum Cu and oxidase (ceruloplasmin) activity when measured by the techniques described herein. (2) Although hypercupremia and CRP generally occurred together, there were sufficient exceptions to indicate that these 2 serum components are not necessarily interdependent. (3) Further work is needed to establish what other factors are involved with the regulation of serum Cu levels.

2 *Biochemistry*

STUDIES ON THE CRYOPRECIPITABILITY OF CRYOGLOBULINS. Demetrios A. Rigas and Robert D. Koler.* Div. of Exp. Med., Univ. of Oregon Medical School, Portland, Oregon.

Cryoglobulins precipitate in the cold and redissolve at 37°C. The reversibility of this phenomenon indicates that it does not involve covalent bonds. Hydrogen bonding participates in the cryoprecipitation since 1 M urea abolishes it in four cryoglobulins studied. In one case, participation of Coulomb forces was also demonstrated. This cryoglobulin precipitates in the cold as a colorless, transparent, highly viscous material. Optimum conditions are pH 7.0 and 20 mM NaCl. Its solubility increases on both sides of these values and with increasing temperature. Amines and basic amino acids effectively prevent its cryoprecipitation. Their effectiveness increases with the basic character and the length of the hydrocarbon chain. Thus, the minimum effective concentration of ethylamine and lysine is 6 mM; of norvalamine, 3 mM; of putrescine, 1.5 mM. The increase in the isoelectric point by these substances and the return of cryoprecipitability following their removal indicate a reversible association with the protein. Van der Waals' forces and perhaps hydrogen bonding are important in stabilizing this complex. The copolymerization of this cryoglobulin with fibrin monomers results in a rigid gel-like non-retracting clot. This is also prevented by 6 mM lysine, normal clotting occurring in its presence.

3 *Biochemistry*

FRACTIONATION OF THE HUMAN Ba-α_2-GLYCOPROTEINS BY STARCH GEL ELECTROPHORESIS. K. Schmid, W. Bürgi,* and K. Dus.* Mass. General Hosp. and Harvard Med. School, Boston, Mass.

The low molecular weight (3S) human plasma glycoproteins (Cohn's Fraction VI)have been fractionated as described earlier. Terminal amino acid analysis of the Ba^{++}-precipitable α_2-glycoproteins yielded Thr and Ala as N-terminal and Leu and Thr as C-terminal amino acids, suggesting the presence of probably two similar proteins with one polypeptide chain each. The aim of this study was to separate these two glycoproteins from each other. Successful separation was achieved by starch-gel electrophoresis at pH 8.6 in tris-borate-EDTA buffer. Both the slow and the fast moving component, following their elution, exhibited the same electrophoretic mobilities on re-electrophoresis. Removal of starch was obtained by dialysis and subsequent addition of NaCl, and finally by digestion with amylase. Preliminary N-terminal amino acid analysis of the faster moving Ba-α_2-glycoprotein indicated the presence of Thr only. (Supported by H.H. Whitney Foundation and Grant A-2288 of Natl. Insts. of Health.)

4 *Biochemistry*

PAPAIN CLEAVAGE OF HUMAN AND RABBIT γ-GLOBULINS. Frank W. Putnam, Shu-hsi Hsiao,* and Margaret Tan.* Dept. of Biochemistry, College of Medicine, Univ. of Florida, Gainesville, Fla.

The rate and mechanism of cleavage of γ-globulins of various species by mercuripapain were studied by chromatography, ultracentrifugation and starch gel electrophoresis with use of pCMB to stop the reaction. In all cases papain resistant 3.5S fragments are formed but the rate and nature of the products depend on the species. Within 10 min. 80% of human γ-globulin is cleaved to 3.5S fragments which at longer times are separable into two fractions (A and B). These differ in amino acid composition, B containing more dicarboxylic and less hydroxy amino acids. Peptides are also split off during the initial cleavage but no free amino acids. Rabbit γ-globulin, unlike human, initially yields equal proportions of three 3.5S fractions (I, II, and III), which are resistant to papain and are separable chromatographically and electrophoretically. The amino acid composition of purified Fraction II is independent of time of incubation from 1 to 16 hours. Although this work supports the hypothesis that rabbit antibody is composed of three subunits having specific biological functions (Porter, R.R., Biochem. J. 73, 119 (1959), human γ-globulin appears to differ in structure. The papain cleavage of γ-globulins seems to involve specific sites with bonds that are as sensitive as those in synthetic substrates.

5 *Immunology*

ANTIGENIC DIFFERENCES BETWEEN THE 7S AND 19S COMPONENTS OF NORMAL HUMAN γ GLOBULIN AND THE 19S MONOMERS. E.C.Franklin and C.A.Reisner*. New York Univ.Coll.of Med., New York.

Previous studies have demonstrated significant cross reaction between the 7S and 19S components of normal human γ globulin, as well as antigenic specificity for the 19S component.Similarly, the 7S component has specific antigenic groups not present in the 19S fraction. Comparison by double diffusion in agar of the reaction of highly purified preparations of 7S and 19S γ globulins with an antiserum to 7S γ globulin showed a spur on the 7S line. Following complete absorption of this antiserum with 19S γ globulin, it still reacted with the 7S fraction to give a line which fused with a line between 7S γ globulin and the original antiserum.

The 7S monomers produced by dissociation of normal 19S γ globulin with mercaptoethanol and dialysis against iodoacetamide, and the reaggregated fractions also lacked antigenic groups present in native 7S and 19S γ globulins. The monomers were separated by stepwise elution on a DEAE column. One fraction appeared at pH 8 (.01M.); the second at pH 6 (.15M.); and a small amount at pH 5 (.3M.). These findings indicate the nonidentity of the 7S γ globulin and the 7S units of the 19S fraction, and further suggest the heterogeneity of the 7S units of the 19S fraction.

Supported by A.R.F. and N.I.A.M.D.(USPHS) Grant A-2594

045. BLOOD–Proteins–Hemoglobin

1 *Biochemistry*

THE NATURE OF THE CHEMICAL ABNORMALITY IN HEMOGLOBIN-G. R. L. Hill, R. T. Swenson*, and H. C. Schwartz*. Depts. of Biochem. and Med., University of Utah, Salt Lake City, Utah.

A chemical abnormality in the structure of hemoglobin-G (Hb-G) has been described (Hill and Schwartz, Nature, 184, 641 (1959). Examination of a tryptic digest of Hb-G showed a single abnormality which is related to the abnormality in hemoglobin-S and the normal sequence in hemoglobin-A:

Hb-A Val.His.Leu.Thr.Pro.Glu.Glu.Lys.
Hb-G Val.His.Leu.Thr.Pro.Glu.Gly.Lys.
Hb-S Val.His.Leu.Thr.Pro.Val.Glu.Lys.

Separation of α- and β-chains of Hb-A and Hb-G showed that the abnormality occurs in the β-chain, the same chain in which the abnormal residue of Hb-S is found (Ingram, Nature, 138, 1795 (1959). Hb-A and Hb-G can be digested with leucine aminopeptidase. Isolation of the degraded hemoglobins and subsequent analysis of the peptides obtained after tryptic digestion, shows that the abnormal peptide is absent. This indicates that the abnormal residue of Hb-G is the 7th from the N-terminus of the β-chains. These results will be discussed in light of the present genetic evidence which indicates that the genes which control synthesis of Hb-G and Hb-S, segregate independently, and in relation to current views of protein synthesis.

2 *Biochemistry*

CHEMICAL BASIS OF THE BOHR EFFECT. Reinhold Benesch and Ruth E. Benesch. Marine Biological Laboratory, Woods Hole.

-SH groups cannot be the source of the protons liberated when hemoglobin is oxygenated, as proposed by Riggs (Nature, 183, 1037,1959), since the two reactive -SH groups of human hemoglobin can be blocked with silver, mercury or iodoacetamide without any change in the Bohr effect. N-ethyl maleimide, however, in agreement with Riggs' observation, reduces the Bohr effect to about one half. Evidence will be presented to show that this inhibition is due to an acylation of two of the oxygen-linked acid groups by the imide portion of the NEM. Model experiments show that NEM can react both with glycine ester and with imidazole at pH 7.3. However, in hemoglobin acylation takes place only if the NEM is first anchored to the -SH groups, since NEM has no influence on the Bohr effect if the -SH groups have been blocked by iodoacetamide. The two reactive -SH groups and two of the oxygen-linked acid groups must thus be in close proximity. Moreover, the two separate reactions which participate in the inhibition of the Bohr effect can be separated by reacting NEM with reduced hemoglobin at pH 7.3. Under these conditions NEM does react with the -SH groups but the Bohr effect remains unaltered. Upon oxygenation, acylation takes place, since the usual inhibition is then observed.

3 *Biochemistry*

THE IDENTIFICATION OF THE OXYGENATION-LINKED ACID GROUPS WITH THE β-CHAIN OF HUMAN HEMOGLOBIN. A. Riggs and M. Wells*. Dept. of Zoology, Univ. of Texas, Austin.

The oxygenation of hemoglobins is associated with the discharge of protons. A comparison of the number of protons dissociated by the hemoglobins of various mammals with the number of mercuric ions which may be immediately bound has led to the identification of the oxygenation-linked acid groups as sulfhydryl groups (Riggs, Nature, 183:1037, 1959). Human hemoglobin binds 2.2-2.3 moles of either mercuric chloride or N-ethyl maleimide; binding by either reagent prevents binding by the other. We have labeled human hemoglobin with C^{14}-N-ethyl maleimide. Human hemoglobin is known to contain two types of polypeptide chain, α and β (Rhinesmith, Schroeder and Pauling, J. Am. Chem. Soc. 79:4682, 1957). We have now separated the chains prepared from labeled hemoglobin using the procedure of Wilson and Smith (Can. J. Biochem. Physiol., 37:405, 1959). The specific activity of the β-chain was found to be 10300 cpm/mg. compared with 312 for the α-chain. Thus at least 97% of the radioactivity is associated with the β-chain. We conclude that the β-chain contains the oxygenation-linked acid groups of human hemoglobin. This implies a special relationship between heme and the β-chain not shared by the α-chain.

4 *Biochemistry*

OXYGEN METABOLISM IN HEMOGLOBIN BREAKDOWN TO BILIVERDIN. F. K. Anan (intr. by E. S. West) Dept. of Biochemistry, Univ. of Oregon Medical School, Portland, Oregon.

An approach to the mechanism of Hb degradation to bile pigments has been attempted, using O^{18} as a tracer. Since the spectrometrically known intermediates are so unstable as to make their purification very difficult the O^{18} incorporation into biliverdin was determined. When the Hb solution was treated at pH 7.6 under O_2^{18} with fractional additions of neutralized ascorbate solution at 53° C for 2 hours, the isolated biliverdin was enriched by approx. 1 atom of O^{18}.

5 *Biochemistry*

THE CHEMICAL DIFFERENCE BETWEEN NORMAL HUMAN HEMOGLOBIN AND HEMOGLOBIN I. Makio Murayama. Natl. Inst. of Arthritis and Metabolic Diseases, Bethesda, Md.

It has been reported (Murayama and Ingram, Nature 183: 1798, 1959) that a specific color reaction on a 'fingerprint' indicated that peptide 23 of Hb I contains tryptophan whereas the corresponding one in Hb A does not. It was found (Ingram, Nature 183:1795, 1959) that this peptide is in the α chain of the hemoglobin molecule. Peptide 23 of Hb A has been analyzed 4 times with an automatic apparatus (Spackman, Stein, and Moore, Anal. Chem., 30:1190, 1958) and found the following: $(Ala)_4$,$(Gly)_3$,$(Glu)_3$,(Val),(Leu),(Arg),(His),(Tyr). In the corresponding peptide of Hb I about 5 more amino acids are found. In the α chain of Hb A there is only one tryptophan containing peptide (Konigsberg, private communication); it is a pentapeptide with lysine as the C-terminal. The composition of the pentapeptide agrees well with the additional amino acids found in peptide 23 of Hb I except that there is aspartic acid in place of lysine. The exchange in this instance is essentially converse to that of Hb A to Hb C, i.e., glutamic acid to lysine (Hunt and Ingram, Nature 181:1062, 1958).

6 *Biochemistry*

BIOSYNTHESIS IN VITRO OF HEMOGLOBIN LABELED WITH IRON-59 IN SICKLE CELL-HEMOGLOBIN C DISEASE. L. M. Kraus and A. P. Kraus (intr. by T. P. Nash, Jr.). City of Memphis Hosp. and U. of Tennessee Medical Units Memphis.

The synthesis of iron-59 labeled hemoglobin S and hemoglobin C during incubation of bone marrow from patients with Sickle Cell-Hemoglobin C Disease has been investigated. Following incubation with iron-59 as ferrous chloride, the radioactivity per unit of hemoglobin from the washed laked cells was determined. Following paper electrophoretic fractionation of the bone marrow hemolysates, from 70 to 90 per cent of the total radioactivity present was found in the hemoglobin fractions. The relationship between per cent distribution of the proteins and of the radioactivity in the two fractionated proteins, hemoglobin S and hemoglobin C, will be discussed. Labeled hemoglobin was not found in non-nucleated, non-reticulated cells. The simultaneous synthesis of hemoglobin S and hemoglobin C from these patients is approximately fivefold greater than the synthesis of hemoglobin A from normal individuals and the synthesis of hemoglobin S from Sickle Cell Anemia patients in crisis and fivefold less than the synthesis of hemoglobin S from patients in the steady state of Sickle Cell Anemia.

7 *Pathology*

IMMUNOLOGIC DIFFERENCES BETWEEN "NORMAL" FETAL AND THALASSEMIA FETAL HEMOGLOBINS. Wm.F. McCormick and R.H. Walker. (intro. by C.C. Erickson) Inst. of Pathology, Univ. of Tenn., Memphis

We have previously demonstrated that "normal" fetal hemoglobin consists of a group of immunologically distinct entities. By applying the same techniques to a group of patients with the Thalassemia syndrome, we have shown that the alkali resistant hemoglobin seen in these people is different from those seen in "normal" infants.

Antibodies to the alkali resistant hemoglobins found in "normal" infants were produced in guinea pigs and rabbits by subcutaneous injection of stroma-free hemoglobin solution with Freund's adjuvant, followed by twelve weekly injections of stroma-free hemoglobin equal to 0.6 Gm. of hemoglobin. Hemoglobin from ten infants was pooled and used as the antigen.

Ouchterlony plates with six peripheral and a central well were made. The central well was filled with the anti-F serum produced in the guinea pigs or the rabbits and the peripheral wells with the alkali resistant ("fetal") hemoglobin to be tested.

The alkali resistant hemoglobins found in our group of 20 cases of Thalassemia syndrome were shown to be immunologically different from those seen in 100 normal infants.

046. BLOOD–Proteins–Properdin

1 *Immunology*

COMPARISON OF ZYMOSAN AND BACTERIOPHAGE ASSAYS IN SERUM PROPERDIN DETERMINATIONS IN NORMAL SUBJECTS AND PATIENTS WITH LEUKEMIA AND LYMPHOMA. A.L. Baltch,* A. Hassirdjian,* W. Osborne* and P. Bunn* (intr. by P. Wehrle). State Univ. of N.Y., Med. Col. at Syracuse, N.Y.

Serum properdin titers were studied in 100 normal subjects, 64 patients with leukemia and 80 patients with lymphoma. The mean properdin titer in normals was 4 U/ml. using zymosan technic and 9 U/ml. using phage (E. coli T_2r+) technic. As determined by the zymosan assay the mean properdin titer was low (< 2 U/ml.) in chronic lymphocytic, myelogenous leukemia and Hodgkin's disease (active and inactive). As determined by the phage technic the mean properdin titer was low (< 2 U/ml.) in chronic lymphocytic leukemia and active Hodgkin's disease; normal or high titers were found in chronic myelogenous leukemia and inactive Hodgkin's disease. In reticulum cell and lymphosarcoma the mean titer was normal using both assays. Zymosan and phage assays showed poor quantitative agreement when sera of the same subjects were studied using both assays. Lack of better agreement between the 2 assays suggests the possibilities that properdin is not a single substance, or that factors other than properdin, complement and Mg^{++} may influence the titers. (Supported by a grant from USPHS).

2 *Immunology*

ANTIBODY REQUIREMENT FOR INTERACTION OF PROPERDIN AND BACTERIA. Ralph J. Wedgwood, Western Reserve University School of Medicine, Cleveland, Ohio.

In normal serum properdin participates in certain bacteriolytic activities and in the inactivation of C'3 by cell wall stroma. Some suggest that the activity of properdin is due to antibody. Current data indicate that both properdin and antibody-like substances are required for bacteriolysis and inactivation of C'3. Requirements for both antibody and properdin in lysis of properdin-sensitive bacterial strains can be shown by differential cross-absorption of serum with large amounts of cell wall. Sequential studies suggest: 1) Bact. + Ab. → Bact. Ab. complex; 2) Bact. Ab. complex + properdin → Bact. Ab. P. complex; 3) Bact. Ab. P. complex + C' → Lysis. Requirement for antibody-like substance and properdin can also be shown for inactivation of C'3. Absorption of serum at 0°C with cell wall stroma interferes with C'3 inactivation by homologous but not antigenically heterologous cell wall. The substance absorbed is not properdin (Blum and Lepow, Fed. Proc. 18, 559, 1959). Interaction of cell wall with the substance resembles Ag-Ab interaction. These observations may perhaps in part reconcile conflicting points of view in this field.

3 *Immunology*

ISOLATION OF A NEW FACTOR IN THE PROPERDIN COMPLEMENT SYSTEM. Myron A. Leon and Arnold Ransby, Jr.* St. Luke's Hospital, Cleveland, Ohio.

A new factor has been isolated from human serum which converts heat inactivated properdin-zymosan complex (iPZ) into the active form (PZ) capable of reaction with C'3. The factor has been isolated, using a combination of alcohol fractionation and elution from a DEAE cellulose column. Essentially no properdin, C'1, C'2, C'3 or C'4 can be measured in the fraction. The kinetics of the reaction of factor with inactivated PZ have been studied. Although the formation of PZ is inhibited by certain sugars and sugar alcohols (Leon, M.A. and Tipper, D.J., A.C.S. Abstracts, Sept., 1959), inhibition does not occur at the step where the new factor reacts with iPZ. (Supported by a grant from the National Institutes of Health).

4 *Immunology*

Nucleotides and Carbohydrates as Effective Inhibitors of Serum Carcinolysis. Robert Willheim, Benedict O'Malley and Paul Fluss. (intr. by J. Parfentjev) Inst. of Appl. Biol. New York and Fairleigh Dickinson University, New Jersey.

Original studies by O'Malley and Osawa in 1958 demonstrated that adenosine-5monophosphate treated cells remained viable after the addition of RNase to the media. There was thus provided a growth model system for the biochemical study of changes in growth acceleration and inhibition. The model system was extended to psychopharmacological agents and A5MP. Studies were conducted to further examine this system as a basis of elucidating fundamental factors in serum carcinolysis. It was found that carcinolysis was most effectively inhibited by adenosine-5-monophosphate as well as a series of nucleotides including ATP, ADP and AMP. Other inhibiting substances were guanine, ribose and galactose. Glucose did not markedly inhibit carcinolysis. It is concluded that results of the A5MP model system in terms of growth and metabolism are directly related to carcinolysis, thus providing an effective screening system for potentially effective carcinolytic and carcinostatic agents in the chemotherapeutic approach to cancer. Further that a relationship exists between these studies on carcinolysis and the Properdin system, based on the effectiveness of the carbohydrates to inhibit carcinolysis.

047. BODY FLUIDS AND SPACES

1 *Physiology*

THE MEASUREMENT OF PLASMA VOLUME AND OF CAPILLARY PERMEABILITY IN THE TURTLE USING T-1824 AND VARIOUS DEXTRAN FRACTIONS. Robert E. Semple. Dept. Physiology, Queen's Univ., Kingston, Ont.

Painted turtles were injected with solutions that contained T-1824 and one of four narrow dextran fractions which had mean molecular weights ranging from 2,300 to 155,000. Disappearance rates of the dextrans from plasma ranged from 50% to 19%/hr. and when plotted against molecular weight resulted in a curve; mean disappearance rates decreased sharply with increasing molecular weight in the low weight range, much more slowly at the upper end of the molecular weight range under observation. The T-1824 disappeared at a rate that corresponded to the rate that would be predicted for a dextran fraction with a molecular weight of about 66,000. Each dextran fraction and the T-1824 disappeared at rates that were significantly greater than is the case in man, dog or rabbit. Distribution volumes for the highest molecular weight dextran were essentially the same as those for T-1824 (ca. 74 ml./kg.); these volumes are much greater than those observed in the mammals. The high plasma volumes, thus indicated, when combined with the low haematocrits observed in these reptiles, gave calculated blood volumes that were within the usual mammalian range. (Supported by a grant from the National Research Council of Canada.)

2 *Physiology*

CHANGES IN EXCRETION RATES NOT MEDIATED BY VOLUME RECEPTORS. Ernest L. Dobson, Frank J. Kelly* and Frederick P. Mustell*. Donner Laboratory, University of California, Berkeley.

Volume receptors have been postulated to explain the increased rate of salt and water excretion which results when the body fluids are expanded by the infusion of isotonic and iso-oncotic solutions. However, when a volume for volume exchange of dextran for whole blood is carried out, a diuresis and a saluresis occur without a change in blood or extracellular fluid volume. Such a response is not explainable by a volume receptor hypothesis. Furthermore the volume for volume exchange of isotonic saline or Locke's solution for whole blood causes a diuresis with a net decrease in blood volume. These phenomena will be discussed in relation to the blood flow dependent control mechanism which has been proposed for the homeostatic regulation of body fluid volume (Homeostatic Mechanisms, Brookhaven Symposia in Biology No. 10, 197-206 (1957)).

XUM

3 *Physiology*

BODY AND BRAIN ELECTROLYTES AFTER MASSIVE INFUSION OF 5% GLUCOSE OR HYPOTONIC NaCl. J. N. Barker and J. P. Capelli (intr. by W. J. Snape) Dept. Physiol., Jefferson Med. Coll., Phila.

Rats were infused i.v. with 5% glucose (G) or 0.08 M NaCl (Na) at 0.5 ml/100 g/min until death at mean fluid retentions of 50 ml/100 g. In G rats, plasma conc at death were: 302 mosm/l, 43 meq/l Na, 5.9 meq/l K, and 3545 mg% glucose. In Na rats, conc were: 246 mosm/l, 117 meq/l Na, 6.1 meq/l K, and 170 mg% glucose. Mean free H_2O calculated from plasma osmolality was 0.5 ml/100 g in G and 14.7 ml/100 g in Na rats. Under G conditions of isosmolemia, drastic hyponatremia and hyperglycemia, brain H_2O was normal (78.5 ml/100 g) but the volume of distribution of Na was equal to total brain H_2O. Under Na conditions of hyposmolemia, hyperhydremia, and less marked hyponatremia, brain H_2O increased (80.0 ml/100 g) but the volume of distribution of Na remained at the control level of 40% brain H_2O. Brain K was only slightly reduced in both series. If death were due to loss of nerve conduction, different mechanisms of loss must be postulated for G and Na. Death may be attributed to complete loss of the Na gradient in the G series if theories of conduction dependent upon a diffusion gradient for Na are correct. In the Na series, however, the loss of Na gradient is less severe and cannot account for loss of conduction unless markedly potentiated by the hyposmolemia.

4 *Physiology*

RAPID DETERMINATION OF D_2O IN BIOLOGICAL FLUIDS BY THE USE OF THE INFRARED ABSORPTION SPECTROPHOTOMETER. M. D. Turner*, William A. Neely*, and James D. Hardy. The University of Mississippi Medical Center, Jackson, Mississippi.

The laborious analytical procedures previously required for the analysis of D_2O in biological fluids have severely limited the study of body water metabolism. It has been especially difficult to obtain data on the problem of active transfer of water across biological membranes as well as on simple diffusion rates. The strict requirements that must be met for the successful analysis of D_2O by means of the falling drop procedure make this method impractical for most purposes. With the aid of infrared absorption spectrometry we have devised an extremely rapid method by which D_2O may be analyzed in plasma water. One ordinary distillation is required as compared to as many as four distillations for previous methods. Approximately 0.3 gm. of anhydrous $CuSO_4$ is added to five milliliters of plasma containing D_2O. Mix well with glass stirring rod. Place the mixture in a 90° water bath for five minutes. Remove and centrifuge. Place supernatant in an all glass semi-micro still. Dip the tip of a clean glass rod into silicone antifoam and stir into supernatant. Distill. Place distillate into calcium fluoride absorption cells and read against a plasma water blank at 3.98 microns in a double-beam infrared absorption spectrophotometer. The error of this analytical procedure is ± 1%. In conclusion a method has been developed for the rapid preparation and analysis of plasma samples containing D_2O. As many as fifty plasma samples may be processed and analyzed in a single day. (Army Contract DA-49-007-MD-627.)

5 *Biochemistry*

INTRACELLULAR WATER CONCENTRATION IN THE HEART AFTER 700 r OF X-RAYS. W. O. CASTER, Dept. of Physiological Chem., Univ. of Minn., Minneapolis

The estimation of intracellular water and electrolyte concentrations requires knowledge of the extracellular fluid volume. Chloride, sodium, thiocyanate, sucrose, and raffinose spaces were measured in the heart of the rat after exposure to 700 r of total body X-irradiation. Not only do these five estimates of extracellular space differ in absolute terms, but they fail to change in parallel fashion after X-irradiation. Factor analysis can be used to evaluate these different estimates. By using multiple regression methods, the "best" of these estimates were combined to give a single pooled estimate. Using this estimate as a basis for computation, the intracellular water concentration during the first 10 days after total body X-irradiation varied less from day to day than did the total tissue water. In general, the "better" the estimate of extracellular space that was used for the estimation of intracellular water and electrolyte concentrations, the more nearly constant was the intracellular water concentration. This observation suggests that the concentration of water in tissue may provide a useful, if approximate, basis for estimating changes in extracellular space in tissue analysis work.

6 *Pharmacology*

INTRACELLULAR ALKALOSIS PRODUCED BY DESOXYCORTICOSTERONE ACETATE (DCA) IN RAT SKELETAL MUSCLE. C. D. Withrow* and Dixon M. Woodbury. Dept. of Pharmacology, Univ. of Utah, Salt Lake City, Utah.

The pH of skeletal muscle cells of control and DCA-treated rats has been calculated utilizing data derived from determinations of total CO_2 of tissue, plasma pH, and total CO_2 of plasma. Treatment with DCA caused muscle cells to become more alkalotic than those of control animals. An extracellular alkalosis was also observed in the DCA-treated animals. The effect of DCA to cause both an extracellular alkalosis and a skeletal muscle intracellular alkalosis has been observed by us in eviscerated animals as early as six hours after large steroid doses (20 mg/300 gm rat), as well as in intact animals treated with smaller doses (5 mg/rat/day) for four days. These data suggest that the effect of DCA upon cellular hydrogen ion concentration is not necessarily related to its effect on potassium metabolism. (*Former predoctoral fellow, N.I.H.; supported by Grant B-381, N.I.H. and Pharmacology Training Grant 2G-153, N.I.H.)

7 *Pharmacology*

KINETICS OF C^{14}-SUCROSE DISTRIBUTION IN CEREBRAL CORTEX, CEREBROSPINAL FLUID, AND PLASMA OF RATS. Donal J. Reed* and Dixon M. Woodbury. Dept. of Pharmacology, Univ. of Utah, Salt Lake City, Utah.

C^{14}-sucrose was administered to 225-gm nephrectomized rats either intrathecally or intraperitoneally. All animals were killed 24 hrs. after nephrectomy. Radioactivity was measured in cortex, CSF and plasma obtained simultaneously 10 min. to 24 hrs. after injection of sucrose. After systemic injection, plasma sucrose was almost maximal in 10 min. and was approximately constant after 2 hrs. Cortex and CSF sucrose levels were constant after 4 hrs. at 9 and 12%, respectively, of the plasma levels. After intrathecal injection, cortex and plasma sucrose levels reached a maximum at 2 and 4 hrs., respectively. The plasma level remained nearly constant while the cortex and CSF levels decreased to final values of 30 and 40%, respectively, of the plasma levels. It is concluded that the cortex and CSF sucrose levels are in rapid equilibrium. The observed relationship between CSF, cortex, and plasma sucrose levels can be explained by postulating a flow of sucrose-free water from plasma to CSF to cortex to plasma, with a resistance to sucrose passage between cortex and plasma. (*Postdoctoral fellow N.I.H. Supported by Grant B-381, N.I.H. and Nat'l. Neurological Found.)

8 *Pharmacology*

PERMEABILITY OF THE BLOOD-CEREBROSPINAL FLUID BARRIER IN THE DOGFISH TO SULFANILIC ACID AND p-AMINOHIPPURATE. David P. Rall and Charles G. Zubrod, Nat. Cancer Inst., Bethesda, Md. and Mt. Desert Island Bio. Lab., Salisbury Cove, Maine.

Basic to the current hypotheses describing the blood-cerebrospinal fluid barrier is the supposition of a lipoidal barrier, permeable only to unionized molecules. This notion would be strengthened if it could be shown that a totally ionized compound were essentially excluded from CSF while a compound only slightly unionized approached diffusion equilibrium. Sulfanilic acid (SA) and p-aminohippurate (PAH) were compared since SA is totally ionized, while 1 part in 6000 PAH is unionized, at body pH. Constant plasma drug levels were achieved by repeated injections for 96 hours. Three fish were studied with each drug at each time. Plasma, CSF, extradural fluid (EDF), bile, muscle and brain were sampled. The ratios of drug in fluid or tissue divided by mean drug plasma concentrations were CSF, 0.19; EDF, 0.85; bile, 0.54; brain, 0.18; muscle, 0.15. For PAH: CSF, 0.69; EDF, 0.83; bile, 1.74; brain, 0.20; muscle, 0.23. Ratios for SA at 24 and 48 hours were the same as at 96 hours. CSF and bile ratios for PAH were successively higher at 20, 40, 60 and 96 hours, while the ratios for EDF, muscle and brain were constant after 20 hours. These data support the hypothesis of a lipoidal blood-CSF barrier.

9 *Pharmacology*

LEVELS OF CALCIUM, PHOSPHORUS, PROTEIN AND ALKALINE PHOSPHATASE IN EFFUSION FLUIDS AND SERUM. Arthur Feldstein, Joseph Samuelson and Herta Spencer (intr. by Julian Herrmann.) Montefiore Hospital, New York.

Since the mechanism of formation of effusion fluid is poorly understood, a study of the concentration of several constituents in the fluid and in serum were determined. Fifty-eight samples of chest and/or abdominal fluid were collected from 24 patients with neoplastic disease, from 13 with non-neoplastic conditions and from 11 having effusions of undetermined etiology. Concentrations of calcium and nitrogen were determined on all samples and phosphorus, proteins and alkaline phosphatase on most of these. The concentrations of several constituents of the effusion fluid approximate that of the serum. For instance, concentrations of calcium in all effusion fluids were much higher than expected and were almost as high as in serum but did not correlate with the protein content of the effusions. Concentrations of phosphorus were slightly lower and that of alkaline phosphatase appreciably lower than in serum. Although the mechanism of fluid formation in neoplasia may differ from that of non-neoplastic conditions, no appreciable differences of the constituents could be detected in the two types of effusions. (Supported by a grant from the U.S. Public Health Service.)

10 *Pathology*

COMPARISON OF T1824 & I^{131} AS PROTEIN LABELS IN STUDY OF EXTRAVASCULAR SERUM PROTEIN SPACE IN DOGS. Robert D. Coye, Donald Blink* and Erling Kloppedal*. Univ. of Wisconsin Med. School, Madison, Wis.

The extravascular serum protein space of the dog kidney was calculated by comparing the amount of label remaining in the kidney after perfusion with saline with the serum concentration of the label. Albumin was labelled with T1824 by injecting IV 75 mg/Kg. Whole serum and serum proteins electrophoretically separated on starch blocks were also labelled with I^{131}. The injected radio labelled material usually contained less than 1% free I^{131} by TCA precipitation. The labelled proteins circulated in the dog for periods of from 15 minutes to 24 hours. It was found that the T1824 space increased linearly with time. The average hourly accumulation of dye was equivalent to 2.87 ml of serum per 100 ml of renal tissue. Most of the dye appeared in the cortex and frozen unstained sections showed blue granules concentrated in the proximal tubular cells. The medulla contained less blue dye; this dye was not seen on frozen section, but could be expressed from the cut surface by pressure. The I^{131} labelled material rapidly equilibrated with the extravascular space with an average value of 2.64 ml of serum per 100 ml of renal tissue. (Supported by a grant from U.S.P.H., H-3548.)

048. CARBOHYDRATES

1 *Physiology*

HEPATIC GLYCOGENOLYTIC EFFECT OF VASOPRESSIN. Stanley S. Bergen, Jr.,* Robert Sullivan* and James G. Hilton, Dept. of Medicine, St. Luke's Hospital, New York, N. Y.

The effect of vasopressin and oxytocin on hepatic glycogenolysis has been studied in the unanesthetized dog. Twelve mongrel dogs were prepared with polyethylene catheters implanted in the hepatic and portal veins and the aorta. Pure synthetic lysine vasopressin and oxytocin were administered via portal vein in the fasting state. Ten to 60 units of lysine vasopressin elicited an immediate release of glucose from the liver which reached a peak within five to ten minutes with a return to baseline levels by fifteen minutes. Hepatic-portal glucose gradients increased during hepatic glycogenolysis with portal blood glucose levels falling during the initial fifteen minutes after vasopressin administration with subsequent return to baseline levels. The height of the response seemed poorly related to the dose of vasopressin. 60 units caused an increase of 76 mg%, 40 units a rise of 80 mg% and 20 units a rise of 70 mg%. There was no evidence of increased hepatic glycogenolysis following administration of equal doses of synthetic oxytocin. (Supported by USPH Grant A-2416.)

2 *Biochemistry*

POLYSACCHARIDES THAT CONTAIN SIALIC ACID. Guy T. Barry. Univ. of Tenn. Mem. Res. Center, Knoxville, Tenn.

Chemical analysis has revealed that certain strains of Escherichia coli, Salmonella, Citrobacter freundii and Neisseria Group C meningitidis bacteria contain sialic acid. Cross agglutination tests indicate that some of these strains are related and that they may be divided into three serological groups. A comparison was made of the chemical and serological properties of polysaccharides obtained from members of each group. It was found that the three polysaccharides studied- colominic acid from E. coli, Group C hapten from N. meningitidis and a polysaccharide from C. freundii contain different amounts of sialic acid ranging from 36 to 99% and that other substances are in chemical combination with the sialic acid. The Group C hapten and C. freundii polysaccharides yield a precipitin with antibacterial antiserum prepared against the organism from which they are derived and with antisera prepared against those bacteria shown by the agglutination tests to be related to each of the organisms. Thus, the occurrence of the sialic acids in various bacterial polysaccharides in different chemical combinations indicates that these monosaccharides are common constituents of cells. (Supported by a grant from the United States Public Health Service).

3 *Biochemistry*

STUDIES ON THE ENZYMIC DETERMINATION OF GLUCOSE AND AN IMPROVED GLUCOSE-OXIDASE METHOD. Mary E. Washko (intr. by Richard H. McCoy) Presbyterian Hospital, Pittsburgh, Pa.

Although the determination of glucose employing the glucose oxidase-peroxidase (Glucostat) system is well established, existing methods generally suffer from three disadvantages: 1) the enzyme reagent is relatively unstable, 2) the final reaction milieu is frequently buffered insufficiently, and 3) the maximum color of the chromogenic product is not attained. A method alleviating these shortcomings has been developed by incorporating the following modifications: the enzyme reagent is stabilized and buffered by diluting with buffered glycerol in place of water and the maximum color is developed by the addition of a large excess of acid. The improved procedure which will be presented in detail is particularly useful for routine usage. The method has also been employed to reinvestigate the partition of glucose between cellular components and plasma of several mammals including man. In man, the ratio, corpuscular glucose/plasma glucose averages 0.6-0.7. In other mammals this ratio is substantially lower.

4 *Biochemistry*

ISOTOPE FRACTIONATION DURING H_2 FORMATION. M.I. Krichevsky, F.D. Sisler and I. Friedman (introduced by B. Wright). NIH, Bethesda, Md. and U.S. Geol. Survey, Wash., D.C.

A marine pseudomonad produces H_2 depleted in D. Experiments with resting cells and extracts showed that the H_2 is formed by a formic hydrogenlyase (FH) typical of coliform bacteria. FH, but not formic dehydrogenase or hydrogenase, was partially inhibited by 33% D_2O or DCOONa. This indicates that one but not two of the atoms in evolved H_2 was in equilibrium with the medium. Mass spectrographic analysis of the H_2 evolved by growing cells, resting cells, and cell-free extracts showed a D content of about 30 ppm (depleted by a factor of 5). The same is true whether glucose or formate is the substrate. The intracellular H_2O and the cellular H are not significantly depleted in D. It is concluded that the discrimination takes place during formate utilization. Clostridium kluyveri also evolved H_2 depleted in D.

5 *Biochemistry*

PARTIAL PURIFICATION OF DEOXYRIBOSE-5-PHOSPHATE ALDOLASE FROM RAT LIVER. Henry G. Roscoe* and Walter L. Nelson. Dept. of Biochemistry and Nutrition, Cornell Univ., Ithaca, N. Y.

The biosynthesis of deoxyribose in mammalian tissue has not been resolved. Although there is evidence of the direct conversion of ribose to deoxyribose at the nucleoside level (J. Biol. Chem. 202, 635, 1953), there is in vivo (J. Biol. Chem. 234, 246, 1959) and in vitro (J. Biol. Chem. 233, 535, 1958) evidence suggestive of an origin of deoxyribose by the condensation of a 2-carbon piece with a 3-carbon piece. It was therefore of interest to attempt a purification of deoxyribose-5-phosphate aldolase from rat liver. The source of this enzyme was the 100,000xg supernatant fraction obtained from a 25% homogenate of rat liver in isotonic KCl. The 100,000xg supernatant was treated with a saturated solution of $(NH_4)_2SO_4$ and the fraction precipitating between 20% and 30% saturation (F-I) contained most of the recoverable activity and represented a 5-fold purification. F-I was again treated with saturated $(NH_4)_2SO_4$ and the fraction precipitating between 25% and 30% saturation (F-II) represented a 15-fold purification. All fractions thus far studied have a sharp pH optimum at 6.8, in contrast to the bacterial enzyme. F-II shows a partial requirement for aldolase with HDP as substrate. Further properties of DORP aldolase are currently under investigation and will be discussed.

6 *Biochemistry*

OXIDATION OF D-GLUCURONIC ACID TO GLUCARIC ACID BY PHASEOLUS AUREUS SEEDLINGS. D. S. Feingold, G. A. Kessler*, E. F. Neufeld, and W. Z. Hassid. Dept. of Biochemistry, Univ. of California, Berkeley, Calif.

Newly germinated mung bean seedlings from which the cotyledons had been removed were incubated from 15 minutes to 6 hours in 0.5×10^{-3} M C^{14}-labeled sodium D-glucuronate (37 μc/μmole). Examination of the main reaction product present in the external solution showed that it had the electrophoretic mobility of a hexaric acid at pH 3.6, 5.8, and 9.0. Incomplete oxidation of this acid with sodium periodate produced radioactive glyoxylate and formate, whereas oxidation in the presence of excess sodium periodate yielded CO_2 and formate. The sugar alcohol obtained by treatment of the hexaric acid with 5% HCl in anhydrous methanol followed by reduction with sodium borohydride was identical with glucitol when examined by chromatographic and electrophoretic procedures. This indicates that the D-glucuronic acid is oxidized by the plant to glucaric acid. Labeled glucaric acid, identified by the same criteria, was also formed from C^{14}-D-glucuronic acid by mitochondrial preparations from mung bean seedlings. (Supported in part by a grant (A-1418) from the U.S. Public Health Service and a contract with the Atomic Energy Commission).

7 *Biochemistry*

GLUCOSE AND GALACTOSE METABOLISM IN RABBIT POLYMORPHONUCLEAR LEUKOCYTES. R. Stjernholm* and E. P. Noble* (intr. by C. Cooper) Dept. of Biochemistry, Western Reserve University, Cleveland, Ohio.

Leukocytes were incubated with serum containing C^{14} labeled hexose. Glycogen from the cells and lactate of the medium were isolated, purified and the C^{14} distribution was determined. About 80% of the glucose-1- or -2-C^{14} entered the glycogen without randomization as compared to 90% for galactose-1- or -2-C^{14}, indicating that galactose is incorporated more directly than glucose. The remaining C^{14} activity in the glycogen was mainly accounted for in C-6 and C-5 respectively. It is proposed that the C^{14} entered these positions via transaldolase exchange (Wood et al. Fed. Proc., 18, 1403 (1959)). This reaction was postulated to explain the asymmetric labeling of glycogen of leukocytes from 2- and 3-carbon substrates (Noble et al. Fed. Proc., 18, 1164 (1959)). Glucose-6-C^{14} gave 98% of the activity in the C-6 of glycogen, which is in accord with this exchange mechanism. The lactate was labeled the same from either glucose or galactose indicating similar metabolism of the two substrates at the triose level.

8 *Biochemistry*

L-XYLULOSE METABOLISM BY AEROBACTER AEROGENES. R. L. Anderson (intr. by H.M.Sell.) Dept. Agr. Chem., Mich. State Univ., E. Lansing, Mich.

A. aerogenes grows readily on L-arabitol and grows after 4 to 14 days on L-xylose or xylitol. Extracts from an L-xylose-positive mutant contained metal-activated isomerases for L-xylose, L-lyxose, D-arabinose, L-rhamnose and L-fucose. L-xylulokinase was demonstrated by two methods. The presence of an L-xylulose-5-phosphate 4-epimerase was indicated from the following: (a) L-xylulose (Xu) + ATP yielded sedoheptulose, glucose, and fructose phosphates; (b) L-Xu + ATP, phosphoketolase, and aldolase yielded fructose diP; (c) absence of L-ribulose-5-P 4-epimerase; (d) absence of D- or L-Xu reductases. Extracts of L-arabitol- or xylitol-grown cells contain pyridine nucleotide-linked reductases for D- and L-Xu. With L-arabitol extracts, oxidation of L-arabitol by lactic dehydrogenase, DPN, and pyruvate yielded L-Xu. Also, L-xylulokinase is present. Thus L-Xu can be formed from L-arabitol and presumably xylitol by dehydrogenation, and from L-lyxose and L-xylose by isomerization. Further L-Xu metabolism appears to involve phosphorylation and the conversion to a D-pentose phosphate presumably by a new L-Xu-5-P 4-epimerase. Thus L-Xu metabolism in this bacterium involves different reactions than observed in plants and animals.

9 *Biochemistry*

PATHWAYS OF FORMATION OF GLUCOSE FROM C^{14}-PYRUVATE AND C^{14}-LACTATE IN HUMANS. Walton W. Shreeve and Roger C. DeMeutter*. Brookhaven National Laboratory, Upton, N. Y.

Distribution of C^{14} in blood glucose has been analyzed in approx. 15 studies using 2- and 3-C^{14}-labeled pyruvate and lactate (DL) in diabetic patients either untreated or after acute dosage with insulin or tolbutamide. Glucose degradation started with fermentation by Leuconostoc mesenteroides. With either 2- or 3-labeled precursors the C^{14} was in most cases distributed much more evenly between the 1 and 2 carbons of glucose than between the 5 and 6 carbons. Carbons 1 to 3 contained 50 to 75% as much total C^{14} as carbons 4 to 6. A typical pattern of labeling is as follows: from 2-C^{14}-pyruvate, C-1 = 59, C-2 = 60, C-3 = 14, C-4 = 10, C-5 = 100, C-6 = 61. Treatment did not particularly affect the C^{14} pattern. While the distribution in carbons 4 to 6 indicates the E-M pathway after CO_2 fixation, the departure from mirror-image symmetry between the two halves of the glucose molecule suggests other metabolic reactions. Rearrangement of glucose carbons 1 to 3 by the pentose cycle (Wood, H.G. and Katz, J., J.B.C. 233, 1279, 1958) does not appear to account for the degree of asymmetry. (Supported by the Atomic Energy Commission.)

10 *Biochemistry*

STUDIES ON THE MECHANISM OF ACTION OF TRANSALDOLASE. R. Venkataraman*, A.G. Datta* and E. Racker. The Public Health Research Institute of the City of New York, New York 9, N.Y.

Highly purified preparations of transaldolase (TA) catalyze a transfer reaction from D fructose-6-P (F-6-P) to L glyceraldehyde-3-P (G-3-P) yielding D G-3-P and a new hexose phosphate. The latter was not oxidized by the action of glucose-6-P (G-6-P) dehydrogenase + G-6-P isomerase. The free sugar has the same R_f value as L sorbose. The sugar-P was tentatively identified as sorbose-6-P (S-6-P). The reaction is reversible, since with TA F-6-P is formed from D G-3-P and the isolated sugar-P. Synthetic L S-6-P (kindly supplied by Dr. H.A. Lardy) also yielded F-6-P under these conditions. The reaction was therefore written as: D F-6-P + L G-3-P $\rightleftharpoons$ L S-6-P + D G-3-P and was used for the quantitative determination of D F-6-P, L S-6-P and L G-3-P by coupling the reaction to either G-6-P dehydrogenase or G-3-P dehydrogenase. The preparation of L G-3-P from DL G-3-P by enzymatic removal of D G-3-P with G-3-P dehydrogenase will be described. Similar to the incorporation of C^{14} labeled glycolaldehyde into F-6-P by transketolase, C^{14} labeled dihydroxyacetone (DHA) was slowly incorporated into F-6-P in the presence of TA, suggesting the formation of a dissociable DHA-enzyme as an intermediate in the TA catalyzed reaction. Experiments on the isolation of a DHA-enzyme on CM-cellulose will be reported.

049. CARBOHYDRATES–Abnormal Metabolism

11 *Biochemistry*

STIMULATORY EFFECT OF DRUGS ON THE GLUCURONIC ACID PATHWAY. J. J. Burns, A. H. Conney* and C. Evans*. Natl. Heart Inst., Bethesda, Md. and Goldwater Mem. Hosp., N.Y.

Administration of barbital or Chloretone to rats leads to a marked increase in the metabolism of glucose-1-C^{14} or galactose-1-C^{14} to D-glucuronic, L-gulonic and L-ascorbic acids via the glucuronic acid pathway which was shown by measuring the incorporation of C^{14} into urinary free D-glucuronic, L-gulonic and L-ascorbic acids. About 4% of a dose of galactose-1-C^{14} was recovered as these urinary products in drug treated rats compared to less than 0.2% of the dose in normal rats. Pretreatment of rats with Chloretone results in increased ability of liver homogenate to convert D-galactose to D-glucuronic acid, whereas no enhanced conversion of D-glucuronic acid to L-ascorbic acid was detected. The stimulatory effect on the glucuronic acid pathway is shown by many drugs having completely unrelated chemical and pharmacological properties as tested by their ability to stimulate ascorbic acid synthesis. Included among these are phenylbutazone, aminopyrine, chlorcyclizine, Benadryl, orphenadrine, 3,4-benzpyrene and 3-methylcholanthrene. The possibility that this adaptive response may represent a protective mechanism on the part of the body to foreign compounds is under study.

12 *Immunology*

A CRITICAL ANALYSIS OF THE METABOLIC TISSUE SLICE PROCEDURE. Joseph Cascarano (intr. by H. Green) New York University-Bellevue Medical Center, N.Y.

Although the limiting factors in the metabolic tissue slice procedure have been clearly defined, confusing results obtained with this approach indicate that certain of the necessary considerations are not being satisfied. The tetrazolium technique provides a direct means of exploring the complexities of this procedure since the actual depth of penetration of the colored reaction product can be readily determined by histological inspection. In addition the reduced salt or formazan can be extracted for quantitative purposes. The data show that experimental conditions which either increased or decreased tissue activity, concomitantly altered the penetration of tetrazolium salt. Quantitative measurements which did not take into account the altered penetration were found to be in considerable error. With the conventional respiratory studies, alteration in the depth of penetration of oxygen, under circumstances of changes in metabolism, cannot be readily determined. A mathematical formula has been developed to correct for the incomplete penetration of materials. This would make possible greater standardization of the procedure as well as a better realization of its full potential. (Supported by a grant from the U.S.P.H.S. H-4298).

13 *Immunology*

SOME PHYSICOCHEMICAL PROPERTIES OF ELECTROPHORETICALLY PURIFIED VI ANTIGEN. F. G. Jarvis, M. T. Mesenko and T. D. Perrine (intr. by C. L. Larson) Rocky Mountain Laboratory, Hamilton, Montana.

The Vi antigen from Escherichia (Citrobacter) freundii 6396/38 cells was purified by ether extraction, acetone precipitation and continuous flow curtain electrophoresis. The final biologically active product is essentially homogeneous as judged by various criteria. Viscosity and sedimentation characteristics of the purified antigen have been determined. Infrared spectrophotometry has indicated the presence of a labile carbonyl group on the molecule. Loss of this group by mild alkaline or acid treatment was accompanied by loss of major biological activities of the antigen. Details of the studies concerning viscosity, sedimentation rate and infrared absorption behavior will be presented in this paper.

1 *Biochemistry*

FUNCTION OF INOSITOL IN THE BIOSYNTHESIS OF YEAST CELL-WALL POLYSACCHARIDES. A. K. Ghosh,* F. C. Charalampous and Y. Sison.* Univ. of Pennsylvania.

The function of inositol in the multiplication of Saccharomyces Carlsbergensis (an inositol requiring strain) was studied by following the progressive morphological and biochemical aberrations resulting from inositol deficiency. Cells cultured in inositol deficient media exhibit a slow rate of multiplication culminating in the formation of large multicellular structures indicative of incomplete separation. Parallel chemical and biochemical measurements performed with these cells revealed a marked and progressive increase of the glucan component and a smaller decrease of the mannan component of the cell wall. The glucan to mannan ratio of the inositol deficient cells was three times that of the inositol supplemented cells. The DNA, RNA, protein, and soluble carbohydrate, content of the cells was not significantly influenced by inositol deficiency. Similarly the ability of the cells to oxidize glucose, acetate and ethanol as well as the amount of respiratory enzymes and oxido-reduction coenzymes were unaffected by inositol deficiency. The mechanism of action of inositol in the biosynthesis of cell wall polysaccharides and in cellular differentiation will be discussed. (Supported by a grant from the Amer. Cancer Soc.)

2 *Biochemistry*

STUDY OF A SERUM FACTOR IN SCHIZOPHRENIA. Charles Frohman, Kenneth Latham*, Norbert Czajkowski*, Peter Beckett*, and Jacques Gottlieb*. Lafayette Clinic and Dept. Physiol. Chem. Wayne State U. Coll. Med. Detroit, Mich.

Previous work has shown that the metabolism of chicken erythrocytes in serum from schizophrenic subjects is different from that in serum from controls. Both the relative rates of production of labeled CO_2 from 1 and 6 labeled glucose and the ratio of lactate to pyruvate produced are affected by serum from the patients. (Fed. Proc. 18, 231, 1959) The serum was separated into protein and non-protein fractions by ultrafiltration. The protein portion contained the fraction affecting chicken cell metabolism. The proteins were then separated into 22 fractions by curtain electrophoresis at 4°C in barbital buffer at pH 8.5. Fraction No. 4 consisting of slow moving beta globulins contained the factor. This fraction was further separated by curtain electrophoresis at 4°C. in ammonium acetate buffer at pH 6.9. The active fraction from this separation was chromatographed on D.E.A.E. cellulose ion exchange resin to further purify the fraction. The characteristics of the resulting active factor will be presented.

3 *Biochemistry*

STUDIES WITH 6-DEOXY-6-FLUOROGLUCOSE AND EHRLICH ASCITES CELLS. Charles J. Stewart* and Arne N. Wick. San Diego State College, San Diego, California.

It has been suggested that 6-deoxy-6-fluoroglucose (6FG) inhibits the oxidation of glucose by interfering with a cell entry mechanism in yeast (Blakely and Boyer, Biochim. et Biophys. Acta 16 576 (1955)) and kidney tissue (Serif and Wick, J. Biol. Chem. 233 559 (1958)). In Ehrlich Ascites cells glucose entry is not rate limiting (Crane et al, J. Biol. Chem. 224 649 (1957)). If 6FG acts by inhibiting cell penetration, inhibition might not be expected in Ascites cells. The cells were preincubated for 30 min. with 6FG, ranging in concentration from 0.01 to 0.12 M, before uniformly labeled glucose (0.01 M) was added and incubation continued for an additional 2 hrs. Respiratory CO_2 was collected and counted. 6FG did not inhibit glucose oxidation in the Ascites cells under these conditions. These results support the hypothesis that 6FG inhibits a glucose cell entry mechanism in those tissues where cell entry is rate limiting. (Supported by Grant No. 2949 from the U.S. Public Health Service)

4 *Pharmacology*

DENATURED ETHANOL (SOLOX^{R}) HYPOGLYCEMIA. W. C. Clark, J. E. Wilson* and H. R. Hulpieu. Indiana Univ. School of Med., Indianapolis, Ind.

Ingestion of denatured ethanol (Solox, "Smoke") has been reported to result in profound hypoglycemia (Hammack, J.A.M.A. 165:24, 1957; Gadsden, J.A.M.A. 168:1220, 1958). Alcoholic beverages may also produce a hypoglycemia (Glatt, Brit. M. J. 1:973, 1955; Bottura, Rev. Hosp. Clin. Sao Paulo. V:115, 1950). The above studies were with chronic alcoholic patients who were probably vitamin deficient. Attempts to produce this phenomenon in dogs (Brown, J.A.M.A. 117:12, 1941) and in rabbits (Bennett, Vet. Adm. Tech. B., T. B. 10-89, 6:6, 1953) were unsuccessful. We (Quart. J. Stud. Alc. 15:189, 1954) observed hypoglycemia after ethanol administration to dogs previously treated with oxythiamine. In the present study components of Solox were investigated for toxicity and hypoglycemic activity. Solox or ethanol in large daily doses produced hypoglycemia only if food was refused or liver damage resulted. Withholding food hastened the appearance of hypoglycemia which was not prevented by thiamine. Ethyl acetate, methyl isobutyl ketone and ethanol were the constituents most active in producing hypoglycemia. (Supported in part by USPHS Grant A-2278)

5 *Pharmacology*

ALTERATION OF CARBOHYDRATE METABOLISM BY ETHANOL IN THE THIAMINE DEFICIENT ANIMAL. James E. Wilson*, William C. Clark and H. R. Hulpieu. Dept. of Pharmacology, Indiana Univ. School of Medicine, Indianapolis, Ind.

A profound fall in blood glucose was observed after ethanol administration to dogs pretreated with large doses of oxythiamine (Hulpieu et al., Quart. J. Stud. Alcohol 15: 189, 1954). In the present study, a hypoglycemia could be evoked in the dog and cat, but not in the rat, mouse or pigeon. In the dog, a schedule of three 2 mg/kg doses of oxythiamine at 3-hour intervals followed by 1 gm/kg ethanol 15 hours later gave the most consistent results. Either agent given alone caused only a slight fall in blood glucose, but liver glycogen was reduced to less than 20% of saline controls. Combined administration produced severe hypoglycemia and almost complete depletion of liver glycogen stores. In the rat, a lowering of liver glycogen occurred, but hypoglycemia did not develop. Blood pyruvate levels were employed as an indicator of thiamine deficiency. The rat showed smaller rises in blood pyruvate than either the cat or dog. (Supported in part by USPHS Grant A-2278).

6 *Nutrition*

EFFECT OF HYPOPHYSECTOMY ON INSULIN SENSITIVITY OF THE CHICKEN. T. Koike*, Y. Matsumura* and S. Lepkovsky. Dept. of Poultry Husbandry, Univ. of Calif., Berkeley, Calif.

The apparent ability of several avian species to carry on normal carbohydrate metabolism in the absence of the pancreas has been well established although their response to insulin administration is qualitatively similar to that found in many mammals. In view of these findings, it is of interest to determine whether hypophysectomy in the chicken would, as in mammals, result in insulin hypersensitivity. Normal, pancreatectomized and hypophysectomized White Leghorn chickens (fasted and fed) were used in the present study. Blood sugar determinations were done at intervals up to 3 hours, and in some cases, to 5 hours after the intravenous injection of graded doses of insulin (0.1, 1, 5 and 10 units/Kg). No great differences could be detected among the 3 groups of birds at doses of 0.1 or 1 unit/Kg. The pancreatectomized and hypophysectomized chickens seemed a little more sensitive to insulin than the intact birds. At levels of 5 units/Kg the hypophysectomized group exhibited a greater and more prolonged hypoglycemia and at 10 units/Kg the response to insulin was further exaggerated. The results indicate that insulin sensitivity in the chicken is related to the anterior pituitary as found in mammals, though there may be some differences in detail.

050. CARBOHYDRATES–Glycogen

1 *Physiology*

DURATION OF GLUCAGON ACTION. Joseph E. Sokal (Roswell Park Memorial Institute, Buffalo, New York).

While blood glucose determinations may indicate the time of onset of glucagon action after administration of this hormone, they fail to measure the duration of glucagon action. An experimental technique for determining the duration of glucagon effect has been developed, based on the fact that glucagon inhibits hepatic glycogen deposition. Fasted rats, whose liver glycogen stores have been depleted, are given injections of glucagon at various time intervals before administration of a large glucose load. Liver glycogen is determined at standard time intervals after glucose administration. The delay in liver glycogen deposition, as compared to controls, measures the duration of glucagon action. Intravenous or intraperitoneal glucagon doses of 0.2-0.4 mg/kg completely inhibited glycogen deposition for about 2 hours; subcutaneous injections produced a somewhat longer effect. Increasing the dose to 1.0 mg/kg resulted in distinct prolongation of effect, especially when injection was subcutaneous. In some experiments, glycogen deposition was inhibited for 6 hours. Zinc glucagon produced more prolonged effects than glucagon.

2 *Biochemistry*

COMPARATIVE STUDIES OF HEART AND LIVER GLYCOGEN IN FASTING AND FED RATS. Joseph H. Roe, J. Martyn Bailey*, Benjamin W. Smith* and R. Richard Gray*. Dept. of Biochemistry, George Washington Univ. School of Med., Washington, D. C.

Glycogen fractions extracted from heart muscle by four homogenizations with trichloroacetic acid (TCA) and by boiling the residue after TCA extraction with 30 per cent KOH (residual glycogen) were compared. Average values for TCA-extracted and residual heart glycogen were 57 and 43 per cent of the total glycogen, respectively. TCA-extracted, but not residual, heart glycogen increased significantly during fasting. Previous studies of liver glycogen showed the per cent of total glycogen extracted by TCA and by KOH differed widely in fasting and fed animals. Iodine absorption studies showed an increase in the glucose chain length of heart glycogen as a result of fasting, whereas the chain length of liver glycogen was increased by feeding. TCA-extracted glycogen showed a non-glucose component when analyzed by anthrone, glucose oxidase and copper reduction methods. The non-glucose component had an ultraviolet absorption similar to that of nucleic acid, was present in considerably greater amounts in heart glycogen than in liver glycogen, and the amount could be decreased by repeated solution in TCA followed by alcohol reprecipitation.

051. CARBOHYDRATES–Monosaccharides

1 *Biochemistry*

3-KETOARABOHEPTOSE PHOSPHATE: A PRODUCT OF G6P METABOLISM Hsien-Gieh Sie*, Vijai N. Nigam* and William H. Fishman, Tufts University School of Medicine and the New England Center Hospital, Boston, Mass.

This new heptulose was isolated from an incubation mixture of G6P plus rat liver supernatant and was converted to the tetrabenzoate derivative of its anhydride (J.A.C.S. 81, 6083, 1959). The proposed structure is based on the following; periodate oxidation of the anhydride splits off HCHO, alkaline oxidation and ferric acetate -H_2O_2 degradation of the free heptulose yields arabinose (identified by paper chromatography in two separate solvent systems and as the crystalline diphenylhydrazone, m.p. 202.5°), the free sugar differs in Rf from glucoheptulose and mannoheptulose. Progress towards the laboratory synthesis of 3-KAH anhydrides has been made. 3-KAHP is produced also from G1P and M6P, but not from F1P, Gal6P, 1-P-glucuronate, 6-P-gluconate, deoxyG6P and adenosine. Amongst the biochemical observations are the requirement of the purified enzyme preparations for triose P, an optimum temp. of 43°, inhibition by inorg. P (0.1 M), depletion of the enzyme in certain thiamine-deficient tissues and a wide-spread distribution in Nature. Accordingly, these facts call for investigation of the mechanism of a new pathway of G6P metabolism.

2 *Biochemistry*

ISOLATION OF GALACTOKINASE FROM YEAST. M. R. Heinrich and J. B. Neilands. Dept. of Biochemistry, Univ. of California, Berkeley, California.

Galactokinase is of interest as one of the few enzymes which phosphorylate a sugar on carbon-one and as a direct method of determining the free energy of hydrolysis of ATP. Preliminary to a study of its properties, the enzyme has been isolated from Saccharomyces fragilis C-106, and partially purified. Enzyme activity was assayed by disappearance of free reducing sugar, or by determining the rate of change in color of an indicator due to acid production during phosphorylation of galactose. The enzyme is extracted from air-dried or lyophilized yeast by extraction with 2.2% diammonium phosphate. It is fractionated with ammonium sulfate, an inactive protein precipitate removed at pH 5, and the enzyme adsorbed on calcium phosphate gel at a high gel:protein ratio. After elution from the gel, the enzyme is adsorbed on a column of DEAE cellulose and eluted with ammonium phosphate in a gradient of increasing ionic strength and decreasing pH. Enzyme activity appears in a peak containing approximately 1% of the original protein. The enzyme is stabilized by cysteine and EDTA. It is inactivated at pH 4.5, but is stable to dialysis and freezing near neutral pH.

3 *Biochemistry*

KINASES IN AN N-ACETYL-D-GLUCOSAMINE-REQUIRING LACTOBACILLUS BIFIDUS. P.J. O'Brien*, M.C. Glick* and F. Zilliken. Biochemistry Dept., School of Medicine, Univ. Pennsylvania, Phila.

L. bifidus var. pennsylvanicus requires certain N-containing saccharides for growth. One of these, $[1\text{-}^{14}C]$-α,β-methyl-N-acetyl-D-glucosaminide has previously been shown to be incorporated into muramic acid (O'Brien, Glick and Zilliken, Biochem. Biophys. Acta, in press). In order to follow the metabolic pathway of the incorporation of the growth factors a study was made of phosphorylating systems present in this organism. Unfractionated cell free enzyme preparations exhibit, in the presence of ATP and Mg^{++}, kinase activity toward N-acetyl-D-glucosamine (N-ac-gl) and D-galactose. D-glucose and D-glucosamine are not phosphorylated under these conditions. β-ethyl-N-acetyl-D-glucosaminide and N-acetyl-lactosamine are cleaved to N-ac-gl which is then phosphorylated. Lactose is cleaved to glucose and galactose, the latter compound being phosphorylated. A partially purified enzyme preparation present in the supernatent fluid (1 hr, 102,000 x g) converts N-ac-gl to fructose- and glucose-6-phosphates in the presence of ATP and Mg^{++} at pH 8.1. The particulate fraction of the cell extract exhibits similar activity. At pH 5.2 the latter accumulates N-ac-gl phosphates. This organism possesses little or no hexokinase activity but has galactokinase and N-ac-gl-kinase. (Supported by a grant from the U.S.P.H.S.)

4 *Biochemistry*

STUDIES ON THE BIOSYNTHESIS OF GDP-L-FUCOSE. V. Ginsburg. Natl. Insts. of Health, Bethesda, Md.

GDP-D-mannose is converted to GDP-L-fucose by extracts of A. aerogenes in the presence of TPNH. (J. Am. Chem. Soc., 80, 4426 (1958)). The kinetics of the conversion as measured by following TPNH oxidation spectrophotometrically indicate that reduction is not the initial step in the reaction sequence. In the absence of TPNH, treatment of an incubation mixture of GDP-D-mannose and enzyme with H_2 and rhodium catalyst produced a new ultraviolet absorbing spot corresponding chromatographically to GDP-L-fucose. Colorimetric analysis of the eluted material indicated the presence of 1 μmole of 6-deoxyhexose per μmole of guanosine. However, mild acid hydrolysis liberated GDP and two carbohydrate components, neither of which was fucose. One component, from its chromatographic properties, appears to be 6-deoxymannose while the other component has not been identified. These results suggest the enzymic formation of a 5,6-glycoseen derivative or derivatives from GDP-D-mannose with subsequent chemical reduction to 6-deoxyhexose derivatives.

5 *Biochemistry*

A MAMMALIAN β-KETO ACID REDUCTASE OF UNUSUAL SPECIFICITY. J. Donald Smiley* and Gilbert Ashwell. Natl. Insts. of Health, Bethesda, Md.

Studies on the metabolism of L-idonic acid have revealed that the enzyme previously reported to catalyze the DPN-linked oxidation of L-gulonate to L-xylulose via a β-keto acid [Ashwell, Kanfer and Burns, J. Biol. Chem. 234, 472 (1959)] is active upon all hexonic, pentonic and tetronic sugar acids examined in which the hydroxyl group of the β-carbon has a levo configuration. Similarly, investigation of the reverse reaction with a variety of β-keto acids has demonstrated the formation of β-hydroxy acids in which the hydroxyl group on the third carbon possesses a levo configuration. Thus, 2,3-diketo-L-gulonate is reduced to 2-keto-L-gulonate as identified by ceric sulfate, o-phenylenediamine and borohydride reduction. 2,3-Diketo-D-gluconate and 2,3-diketo-D-glucoheptonate are similarly reduced. Acetoacetate is specifically reduced to L(+)-β-hydroxybutyrate and this isomer is active in the reverse reaction whereas D(-)-β-hydroxybutyrate is inactive. The equilibrium of the reaction favored the reduced product in all cases. The bulk of the activity was retained after treatment of the enzyme with Dowex-1-Cl and no stimulation was observed upon addition of CoA and ATP. The enzyme is present in the soluble fraction of hog, rat, beef, and monkey kidney and liver homogenates.

6 *Biochemistry*

THE METABOLISM OF COLITOSE. Edward C. Heath (intr. by L. H. Louis) Rackham Arthritis Research Unit and Dept. of Bacteriology, Univ. of Michigan, Ann Arbor.

The 3,6-dideoxyhexose components of the bacterial lipopolysaccharides (endotoxins) are primarily responsible for the immunochemical specificities of these substances. Colitose (3,6-dideoxy-L-galactose) is the dideoxyhexose of the lipopolysaccharide produced by Escherichia coli O111 B4 (Luderlutz, et al, Biochem. Z. 330:193,1958).

The metabolism of colitose by this organism was investigated. Fractionation of acid-soluble extracts of the cells on Dowex-1-Cl resin yielded a nucleotide fraction containing colitose. Further purification by charcoal treatment and paper chromatography yielded a product identified as guanosine diphosphate colitose (GDPC); analysis, G:P:C = 1:1.97:0.95. In experiments designed to demonstrate the enzymatic synthesis of GDPC from other guanosine sugar nucleotides (GDP-mannose and GDP-fucose), the formation of GDPC was not detected. However, when crude extracts of E. coli O111 B4 were incubated with C^{14}-glucose 6-phosphate, a radioactive product was formed which migrated identically with authentic colitose in three chromatographic solvent systems. The addition of GTP or reduced pyridine nucleotides to such crude incubation mixtures did not significantly influence the degree of C^{14} incorporation into this compound.

7 *Biochemistry*

ENZYMATIC SYNTHESIS OF N-ACETYLMANNOSAMINE 6-PHOSPHATE. Saul Roseman, Francoise Hayes* and Sudhamoy Ghosh.* Rackham Arthritis Res. Unit and Dept. Biol. Chem., Univ. Michigan, Ann Arbor.

Earlier work (J. Am. Chem. Soc., 80, 497 (1958)) on the metabolism of the sialic acids, showed that N-acetylneuraminic acid (NAN) was synthesized from pyruvate and N-acetylmannosamine (N-AcMm) by an enzyme (NANaldolase) isolated from Clostridium perfringens. While N-AcMm was formed from UDP-acetylglucosamine by rat liver extracts (B.B.A. 29, 653 (1958)), this activity was not detected in bacteria.

In the present studies, the metabolism of N-AcMm was studied with bacterial extracts. Cell-free preparations of Aerobacter cloacae, grown on N-AcMm, catalyzed the interconversion of N-acetylglucosamine 6-phosphate and N-acetylmannosamine 6-phosphate. The enzyme, an epimerase, has been purified 50-fold; the products of the reaction were characterized by paper chromatography and ionophoresis after dephosphorylation. Epimerase activity also was detected in the following organisms after growth on media containing glucose: Cl. perfringens, which utilizes NAN; Escherichia coli K-235, which produces polymers of NAN. The activities of NANaldolase and epimerase in crude extracts of log-phase E coli K-235 is sufficient to account for the rate of NAN synthesis by whole cells of this organism.

XUM

8 *Biochemistry*

ENZYMATIC PROPERTIES OF CARBOXYPEPTIDASE-DEGRADED ALDOLASE. A.H. Mehler and B. Bloom. Natl. Insts. of Health, Bethesda,Md.

Rabbit muscle aldolase was shown by Drechsler, et al. to be altered by carboxypeptidase so that the rate of cleavage of fructose-1,6-diphosphate (HDP) was reduced to 7% while the relatively slow rate of cleavage of fructose-1-phosphate (F-1-P) increased. The ability of the enzyme to form a complex with dihydroxyacetone phosphate (DHAP) as measured spectrophotometrically (Science 126, 1287, 1957) is not altered by carboxypeptidase treatment. An analytical system using glycerophosphate dehydrogenase at pH 10 was developed to permit aldolase kinetics to be measured in both directions as a change in DPNH concentration. This assay is equally applicable to all substrates. In the condensing reaction the DHAP concentration is poised as a function of α-glycerophosphate concentration. The affinity of aldolase for DHAP is not greatly affected by carboxypeptidase, but the inhibition of the condensation reaction by phosphoglyceraldehyde is greatly increased. P-glyceraldehyde inhibits both enzyme preparations in the splitting of HDP. Concentrations of P-glyceraldehyde that inhibit HDP cleavage, in contrast, accelerate the splitting of F-1-P by native aldolase to the rate found with the degraded enzyme; the reaction of the degraded enzyme with F-1-P is not influenced by P-glyceraldehyde.

9 *Biochemistry*

HEPATIC FRUCTOSEDIPHOSPHATASE. Rita Wissel, Rosalyn Gasque, Lillian Griffin, and William L. Byrne. Dept. of Biochemistry, Duke Univ. School of Med., Durham, N.C.

A survey has been made of the fructosediphosphatase activity of rat, rabbit, dog, and beef liver. When crude extracts and partially purified preparations are assayed at pH 6.5 and 9.0 under the standard conditions (2.5×10^{-3}M Mg^{++}, 0.5×10^{-3}M EDTA, 2.5×10^{-3}M fructose-1, 6-diphosphate, 0.025 M Tris, and 0.025M histidine), the various preparations had an activity ratio (pH 6.5/pH 9.0) in the range of 1.4 to 2.0. (Hers and Kusaka, Biochim. Biophys. Acta 11, 427, 1953, and Hass and Byrne, Fed. Proc. 18, 243, 1958). The pH 6.5 activity is preferentially inactivated by a variety of conditions including autolysis at pH 4.5, digestion with papain, and dialysis against 0.01M Tris pH 8.0. The loss of activity at pH 6.5 upon autolysis or papain treatment is concomitant with the previously reported rise in pH 9.0 activity. (Mokrasch and McGilvery, J. Biol. Chem. 221, 149, 1956). Attempts to characterize the response of the activity at pH 6.5 and 9.0 to varying concentrations of Mg^{++}, Mn^{++}, and EDTA have indicated that preparations which are different based on activity ratio also differ in terms of their response to Mg^{++}, Mn^{++}, or EDTA. It appears that "native" fructosediphosphatase can be converted to a number of different forms which retain enzymatic activity.

10 *Biochemistry*

STUDIES ON THE PROPERTIES AND MECHANISM OF ACTION OF VARIOUS ALDOLASES. O. C. Richards* and W. J. Rutter. Div. of Biochem. Dept. of Chem. Univ. of Ill., Urbana.

Drechsler, Boyer, and Kowalsky [J. Biol. Chem., 234, 2627 (1959)] have reported that carboxypeptidase treatment of rabbit muscle aldolase (CP aldolase) reduces the aldol cleavage activity to approximately 7% of the original activity concomitant with the release of 3 moles of tyrosine in COOH terminal positions of the enzyme. Our experiments confirm these observations and have shown in addition that the hydrogen exchange reaction with dihydroxyacetone phosphate catalyzed by CP muscle aldolase is 0.1% of the initial level. CP treatment of bovine liver aldolase reduces the FDP cleavage activity to 40% of the original activity and decreases the exchange activity to 1-2% of the control rate. CP treatment, therefore, dissociates the aldol cleavage activity from the exchange activity and indicates a decisive role of tyrosine in the exchange activity of these mammalian aldolases. Neither the aldol cleavage activity or exchange activity of yeast aldolase is affected by CP treatment. The activity of yeast aldolase is stimulated up to ten fold by the addition of potassium ions. Muscle and liver aldolase are indifferent to potassium ions. These studies indicate basic differences in the yeast aldolase and mammalian aldolases, and emphasize the similarities in the structure and mechanism of action between muscle and liver aldolase.

052. CARBOHYDRATES–Oligosaccharides

1 *Physiology*

ENZYMATIC METABOLISM OF URIDINEDIPHOSPHATE GLUCOSE BY SOLUBLE ENZYMES FROM SPINACH. D. O. Brummond*(intr.by R. A. Shipley) V. A. Hospital and Western Reserve University, Cleveland, O.

Enzymes promoting glycosyl donation from UDPG in the synthesis of di- and polysaccharides have previously been demonstrated in various plant tissues. This report is concerned with a soluble fraction of spinach which, without added activator, converts UDPG to UDP and an unidentified glucose component. A 10-fold increase in specific activity was achieved by centrifugal removal of particulate material, fractional precipitation with $(NH_4)_2SO_4$ and precipitation of inert protein by dialysis at pH 4.5. Chromatographic behavior on IRC-50 columns suggested the presence of more than one active component. No acceleration of UDPG conversion was produced by indole-3-acetic acid, cellobiose, cellulose dextrans or starch. More UDP was produced than reducing sugar. With several hours incubation at high concentrations of UDPG, a precipitate formed which was insoluble in water, 1 N HCl, 1 N NaOH and alcohol. This material gave a reaction for reducing sugar after acetylation and hydrolysis but not prior to such treatment. This suggests the synthesis of an insoluble carbohydrate polymer.

2 *Biochemistry*

LABELING OF LACTOSE PRECURSORS FROM MAMMARY GLAND. R. G. Hansen, H. G. Wood, and G. Peeters*, Michigan State U., Western Reserve U., U. of Ghent.

UDP glucose, UDP galactose, glucose-6-P and other hexose phosphates were isolated from mammary gland preparation immediately after injection of glycerol-1, 3-C^{14}. The specific activity of the glucose and galactose of the nucleotides was 10-fold higher than that of the hexose phosphates. However, the pattern of labeling in the various carbons of all hexose derivatives was the same. The UDP galactose had slightly greater specific activity than did UDP glucose. According to current views on the mode of formation of lactose the hexose phosphates are precursors of the UDP hexoses and their specific activities should be equal to or greater than the UDP hexoses. Two possible explanations are: (1) a particular architecture within the cell which permits compartmentalization of certain of the enzymes and therefore distinct substrate pools, (2) an independent pathway for formation of UDP galactose which does not involve the hexose phosphates isolated.

053. CARDIOVASCULAR SYSTEM

1 *Physiology*

INTEGRATION OF SYMPATHETIC CARDIOACCELERATION IN THE CENTRAL NERVOUS SYSTEM. Clarence N. Peiss. Department of Physiology, Stritch School of Medicine and The Graduate School, Loyola University, Chicago, Illinois.

The hypothesis that sympathetic acceleration of the heart is integrated in the medulla oblongata was investigated. In vagotomized cats under pentobarbital anesthesia, most cardioaccelerator responses elicited by stimulation of the dorsal medulla were of long-latency (12-20 sec.). These responses were largely eliminated by bilateral adrenalectomy. The few short-latency responses were never greater than 15% of the control heart rate. Stimulation of the ventrolateral medulla in the same animals produced large short-latency cardiac acceleration, presumably through activation of hypothalamospinal pathways. Stimulation of the dorsal medulla in vagotomized cats under chloralose anesthesia evoked large cardioaccelerator responses with latencies of 1-5 seconds. These responses appear to be due to activation of afferent pathways to higher levels of the central nervous system, since they are 80-100% eliminated by large electrolytic lesions in the brain stem just caudal to the hypothalamus or by transections at the midcollicular level. The data from these experiments on 96 cats do not support the concept of significant integration of sympathetic cardioacceleration in the medulla of the cat.

2 **Physiology**

THE CENTRAL CIRCULATION IN NEPHROTICS. E. Rapaport*, H. Yamauchi*, J. Green*, E. Brown, and J. Hopper*. U.C. Sch. of Med., and Mt. Zion Hosp., S.F.

The observation that hypovolemic nephrotics are prone to postural hypotension and syncope prompted a hemodynamic study of such patients, including the effects of plasma infusions and exercise. Average blood volume (B.V.) of 10 patients, measured with Cr^{51}, was -18% of that predicted from ht. and pre-edematous wt. (range +9 to -43%). At rest and recumbent, stroke output (S.O.) and cardiac output (C.O.) were generally low. Mean cardiac index was 2.9 L/min/M^2 (range 1.9 to 3.6). Right atrial (R.A.) pressure was between -1 and +5 mm. Hg (av.3), and pulmonary artery (P.A.) pressure was normal. Exercise produced an increase in S.O. and rate in each subject. Mean increase in C.O./100 ml. rise in O_2 consumption was 1.88 L/min., reflecting good myocardial function. Infusions of plasma raised B.V. an average of 17%; in all but 3 studies, final B.V. was within ±10% of predicted. Hematocrits averaged 39% initially and 33% finally. Infusion uniformly resulted in an increase of C.O. (av.58; range 11 to 79%), chiefly due to increased S.O.. Right ventricular filling pressure also rose uniformly (range 1 to 13 mm. Hg). Mean P.A. pressure rose an average 11 mm. Hg (range 5 to 24). The changes in filling pressure seen here were comparable to those found by others after infusion of like volumes into normovolemic subjects; the changes in C.O. were larger. (USPHS grants H-4029 and H-1271)

3 **Physiology**

CARDIOVASCULAR CHANGES DURING CONTINUOUS POSITIVE PRESSURE BREATHING. R. H. Harner*, S. F. Marotta*, and J. P. Marbarger. Aeromedical Laboratory, University of Illinois, Chicago, Illinois.

Lateral blood pressures were recorded in the aortic arch, vena cava, femoral and abdominal arteries, and veins of heparinized mongrel dogs anesthetized with sodium pentobarbital (30 mg./kg., I. P.). Simultaneous measurements of carotid and femoral arterial blood flow were obtained. The application of 18.5 mm. Hg continuous positive pressure (1 hour, ambient air) to the intact dog resulted in an immediate period of apnea which was accompanied by an increase in all venous pressures, as well as a decrease in arterial flow and pressures. While maintaining positive pressure breathing the arterial flow and pressures tended to revert to control levels. Similar cardiovascular alterations were obtained in the double vagotomized animal.

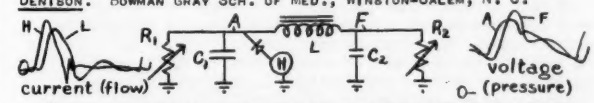
4 **Physiology**

ANTI-EDEMA EFFECT OF INFLAMMATION. D.C. Bowman, Jr.*, D.B. Doemling*, & S.C. Harris. Northwestern U.Dent.Sch., Chicago

A pre-existing inflammation has been shown to inhibit partially the edema elicited by a subsequent irritation. This study was done to determine how soon and for how long this "protection" prevails. The injection of 0.1 cc of 1% $AgNO_3$ into rat ankle joints will provoke quantitatively uniform swellings. In this study, one ankle was so treated and after a selected interval the rat's other ankle was similarly treated. When both legs are irritated at the same time no inhibition of the edematous response occurs until after 48 hours has elapsed. Minimum second-leg edema occurs if first legs are injected 12-15 hours earlier. Increasing the interval between injections of the first and the second-leg reduces the "protection". When 48 hours intervenes, the response of the second-leg proceeds as if no previous inflammation had occurred. Changes observed are not attributed to water imbalance or tolerance to $AgNO_3$. (Supported by NIDH grant # D-400)

5 **Physiology**

AN EXPLANATION OF THE MAJOR FEATURES OF THE ARTERIAL PULSE BY MEANS OF A SIMPLE ELECTRONIC ANALOGY. M. P. Spencer and A. B. Denison. Bowman Gray Sch. of Med., Winston-Salem, N. C.



The left heart is considered to be a stiff current source (constant volume pump) discharging into a tuned circuit which resonates at 3-6 cps through capacitance C_1 & C_2 and inductance L. C_1 represents the lumped compliance of the aortic arch & its branches, and C_2 represents the abdominal aorta & its branches. L is the inertiance of blood in descending aorta, and R_1 & R_2, peripheral resistance. When a current, corresponding to the cardiac ejection pulse H, is fed into the "aortic arch" A, voltages A & F and current L which match pressure & flow in the arteries may be derived by adjustment of $C_{1,2}$, L, & $R_{1,2}$. In order to produce a higher "systolic" voltage at F than at A, $C_2 \cong C_1/10$. We conclude that the systolic pressure in the legs is higher than in the arms because gross compliance of the C_2 arteries is less than that of C_1 arteries. The "dicrotic notch" of the peripheral vessels is the first trough of the resonant wave. The incisura arises from a local 30-60 cps resonant system, including aortic valve & asc. aorta. (Supported by H-2630 and Am. Heart Assn. & N. C. Heart Assn. grants.)

6 **Physiology**

DROWNING TREATED WITH IPPB. J. S. Redding*, G. C. Voigt*, and P. Safar. Baltimore City Hospitals, Baltimore, Md.

A standardized experiment was designed to simulate apneic victims of submersion. The tracheal tube of lightly anesthetized dogs was obstructed (simulating laryngospasm) until breathing movements ceased. After 30 sec. of apnea the lungs were flooded with fresh water or sea water for 30 sec. or apnea was permitted to continue for a comparable period without flooding. After passive drainage (30 sec.) resuscitation was attempted with IPPB. All 5 control dogs (obstruction without flooding) survived. In all 5 fresh water dogs flooding caused a severe rise in venous pressure, bradycardia, hydremia and hemolysis, followed by sudden ventricular fibrillation in spite of IPPB. Sea water flooding (10 dogs) caused severe arterial hypotension, bradycardia and hemoconcentration; IPPB-air (5 dogs) led to partial reoxygenation and partial restoration of circulation; when IPPB-air was discontinued after 10 min. all dogs started breathing spont., but died within a few minutes with pulmonary edema. IPPB-02 (5 dogs) led to complete reoxygenation; when IPPB-02 was discontinued after 3 hours 4 dogs died with pulmonary edema, 1 survived. (Supp. U. S. Army Contr. No. DA-49-007-MD-858).

7 **Pharmacology**

PHARMACOLOGICAL AND CARDIOVASCULAR EFFECTS OF 1-METHYL-LYSERGIC ACID BUTANOLAMIDE BIMALEINATE; A POTENT ANTISEROTONIN COMPOUND. J. H. Trapold, R. Mulford* and D. Grohman*. Pharmacol. Labs, Sandoz Pharmaceuticals, Hanover, New Jersey

1-methyl-lysergic acid butanolamide (UML-491) has been reported (Doepfner and Cerletti, Int.Arch.Allergy 12, 89 (1958)) to be 4.4 times more active than LSD-25 in inhibiting the edema provoked in the rat's paw by serotonin. Additional evidence of UML-491 antiserotonin activity was noted in the present study. Such evidence includes the observation that UML-491 pretreatment (2 mg/kg subcu.) completely protected mice from 48/80 potentiated lethal effects of serotonin whereas it failed to alter the LD50 of 48/80. In barbital anesthetized dogs, a slight biphasic depressor-pressor effect followed by a gradual decrease of 20 to 40 mm Hg in arterial pressure occurred particularly after 2 mg/kg of UML-491. Subsequent injections usually produced a gradual sustained decrease in arterial pressure. All injections of UML-491, even in doses which failed to alter arterial pressure (0.2 mg/kg), potentiated the pressor effect of nicotine (15 γ/kg) frequently augmented the pressor effects of l-epinephrine and norepinephrine (2-3 γ/kg) and either did not alter or antagonized the pressor response to serotonin (25 γ/kg). This altered vascular sensitivity induced by UML-491 is presently under investigation and will be discussed.

8 *Pharmacology*

PHARMACOLOGIC STUDIES ON 8-HALOGENATED THEOPHYLLINES. Arthur Wolpert, Raymond M. Burgison and J. C. Krantz, Jr. Univ. of Maryland, School of Medicine, Baltimore 1, Md.

8-Chloro, bromo, iodo and trifluoromethyl(TRF) theophyllines were compared to theophylline as blood pressure depressants and peripheral vasodilators in anesthetized dogs; as diuretics in rats; as coronary artery vasodilators in the Langendorff isolated rabbit heart preparation; as bronchodilators and tracheal relaxants, antihistaminics and antispasmodics in the guinea pig; and in their action on the frog heart. An attempt was made to correlate blood level concentrations (determined spectrophotometrically) with blood pressure depressant activity in the dog and with diuretic activity in the rat. No correlation was found with blood pressure depressant activity, but fair correlation was found between concentration in the blood and diuretic activity. As a blood pressure depressant, peripheral vasodilator, coronary vasodilator, bronchodilator, tracheal relaxant, and myocardial stimulant, theophylline was found to be more active than its 8-halogenated derivatives. As diuretics, all halogenated compounds were more active than theophylline, the 8-iodo being the most active. The halogenated compounds were bronchoconstrictors and devoid of antihistaminic activity.

9 *Pharmacology*

EFFECT OF CORONARY OCCLUSION ON THE CHRONOTROPIC ACTION OF EPINEPHRINE IN THE MINIATURE PIG. Martin M. Winbury, Lorraine M. Hausler*, Nicholas A. Prioli* and Lester Zitowitz*. Schering Corp., Bloomfield, N. J.

Partial or total occlusion of the anterior descending branch of the left coronary artery was produced under general anesthesia and the animals permitted to recover. There was little change in the heart rate following recovery of the animals; however, some disturbances in rhythm were observed in those with a total occlusion. These consisted of occasional ectopic beats, nodal rhythm or heart block. Sinus rhythm usually returned within a few days. The effect of epinephrine (8γ/kg. i.v.) on the cardiac rate and rhythm was determined in the unanesthetized state before and at intervals (1 to 30 days) after occlusion. Before occlusion epinephrine resulted in sinus bradycardia but after occlusion ectopic tachycardias were observed. On the first post-occlusion day all of the animals (partial or total) showed the ectopic response to epinephrine. The ectopic response disappeared rapidly and by 7 days after occlusion epinephrine resulted in bradycardia. These studies demonstrate that although coronary occlusion does not result in the development of ectopic tachycardias in the pig the myocardium is sensitized to the ectopic provoking action of epinephrine.

10 *Pharmacology*

CARDIOVASCULAR ACTIONS OF 7(N-β-METHYLPHENETHYLAMINOETHYL) THEOPHYLLINE HYDROCHLORIDE (McN-R-720-11). John Yelnosky and Georgia V. Kirkpatrick (intr. by William M. Govier). McNeil Laboratories, Inc., Philadelphia, Pennsylvania.

The principal pharmacologic action of McN-R-720-11 is central nervous system stimulation. This study deals with the cardiovascular actions of McN-R-720-11, which consist primarily of a transient fall in blood pressure and a prolonged increase in the myocardial force of contractions. Typical vasodepressor effects were obtained in anesthetized dogs pretreated with hexamethonium, after bilateral vagotomy and after high transection or complete destruction of the cord indicating a peripheral action. Hypotension was also obtained in dogs pretreated with carbinoxamine, Lilly 20522, and atropine; thus there was no evidence that the hypotensive effect is due to a histaminic, isoproterenol-like, or acetylcholine-like action. Small intra-arterial doses of McN-R-720-11 caused a transient increase in femoral artery blood flow in the denervated hind limb of the dog, indicating that the hypotensive effect is probably due to a direct vasodilator action. Preliminary findings suggest that the positive inotropic effect of McN-R-720-11 is mediated indirectly, since the response is markedly reduced or abolished in dogs pretreated with reserpine.

11 *Pharmacology*

ASSAY OF ANGIOTENSIN ON THE SUPERFUSED GUINEA-PIG ILEUM. M. Brody and L. Beck (intr. by M.H. Seevers), Dept. of Pharmacology, Univ. of Michigan, Ann Arbor.

Angiotensin was assayed by superfusion of the guinea pig ileum. This preparation responds to amounts as small as 0.25 cc of a solution of valine-5-hypertensin-II (Ciba) in a concentration of less than 0.5 ugm/l. This corresponds to approximately 0.0002 Goldblatt Units or 0.1 ng of angiotensin and renders the superfused ileum the most sensitive assay currently available. This angiotensin response is not blocked by agents which specifically block equicontractile responses to acetylcholine, histamine and serotonin. The angiotensin response is potentiated by previous administration of human plasma to the superfused ileum. (Supported by USPHS grant H 2578).

12 *Pharmacology*

EFFECT OF ENDOTOXIN ON CARDIAC OUTPUT AND REFLEX DILATATION IN THE ANESTHETIZED DOG. B. Zimmerman and L. Beck (intr. by M.H. Seevers) Dept. of Pharmacology, Univ. of Michigan, Ann Arbor.

The cardiac filling pressure of intact pentobarbital anesthetized dogs was maintained constant with a pressure stabilizing device throughout the experiments. Endotoxin (Sal. Typ.), 0.5 mg/kg, administered intravenously causes a decrease in heart rate and stroke volume within 20-30 minutes of injection. Blood pressure decreased and tended to parallel the reduction in cardiac output. Within 1-2 hours, the cardiac output and blood pressure return toward normal. Reflex dilatation occurring in response to intravenously administered norepinephrine was tested in the perfused isolated innervated hindlimbs. Reflex dilatation was seldom reduced and more often increased by the endotoxin. Perfusion pressure at constant blood flow tended first to increase and secondarily to decrease as the endotoxin exerted its full effect. Under the conditions of the experiments the observations indicate that the reduction in cardiac output is not the result of peripheral venous pooling and that reflex dilatation is not impaired as a result of endotoxin administration. (Supported by USPHS grant H-2578).

13 *Pharmacology*

PARALLEL DETERMINATION OF DOSE-RESPONSE CURVES FOR THE INOTROPIC AND CHRONOTROPIC ACTIONS OF SUBSTANCES UPON ISOLATED HEART MUSCLE. John R. Blinks (intr. by O. Krayer) Harvard Medical School, Boston, Mass.

Because the force of contraction of isolated heart muscle depends strongly upon the interval between contractions, dose-response curves for the inotropic actions of substances cannot be determined accurately unless the frequency of contraction is controlled. Dose-response curves for the inotropic and chronotropic effects of substances having both actions have been made using strips taken, respectively, from the left and right atria of the same heart (guinea pig or rabbit). The otherwise quiescent strip of left atrium is stimulated electrically; the right atrial strip contains the pacemaker The two are mounted side by side in the same bath, and their isometric contractions are recorded with a pair of strain gauge transducers. Reproducible dose-response curves are usually obtained. For each of the substances, l-epinephrine, levarterenol, and dl-isoproterenol, the inotropic dose-response curve is very similar to the chronotropic. However, the relationship between the two curves for a given sympathomimetic amine varies somewhat with temperature and with the frequency at which the left atrial strip is stimulated. (Supported by USPHS grants H-3738 and H-2205.)

14 *Pharmacology*

REDUCTION OF THE DEPRESSOR EFFECT OF L-EPINEPHRINE. William B. Wood*, Clinton B. Nash*, and Robert A. Woodbury, Div. of Pharmacology, Medical Units, Univ. of Tenn., Memphis.

Dogs anesthetized with sodium pentobarbital (30 mg/kg.) were maintained under artificial ventilation just adequate to eliminate spontaneous breathing movements. Epinephrine reversal was established by the production of adrenergic blockade. The pH of the blood was reduced by administration, through the respirator, of a mixture of 30% carbon dioxide - 70% oxygen. In a few animals succinylcholine chloride was administered by venous infusion to eliminate the exaggerated respiratory efforts produced by the hypercapnia. Periodic blood pH measurements during the administration of the gas mixture gave readings as low as 6.7. The blood pressure effects of l-epinephrine administered intravenously in doses from 1 - 6 ugm/kg. were recorded. With the development of acidosis, the depressor response to epinephrine was markedly reduced or eliminated. As much as a three-fold increase in the dose of epinephrine failed to elicit a depressor response in some animals. A return to air breathing restored the blood pH values to control levels and re-established the depressor response to epinephrine. (Partially supported by a grant from the American Heart Association).

15 *Pharmacology*

THE ACTION OF RA-8 (PERSANTIN) ON CORONARY BLOOD FLOW IN THE ANESTHETIZED DOG. Larry McKnelly* and Theodore R. Sherrod. Dept. of Pharmacology, Univ. of Ill. Coll. of Med., Chicago, Ill.

RA-8, 2,6-bis-(diethanolamino)-4,8-dipiperidino-pyrimido-(5,4-d) pyrimidine, was synthesized in 1951 by Fischer and Roch. This agent proved to be an effective coronary vasodilator in a variety of methods in experimental animals. This study was confined to the action of RA-8 on coronary blood flow in the pentobarbitalized dog, in which a Shipley rotameter was interposed between the femoral artery and the circumflex branch of the left coronary artery. The b.p. was recorded from the left corotid artery by means of a strain guage. Continuous ECG records were made throughout each experiment. In one series of animals RA-8 alone was administered I.V. in a dose of 0.5 mgm/kgm of body weight and in another series the same dose was administered I.V. following the I.V. administration of pitressin, 0.5 unit/kgm. RA-8 alone caused a significant increase in the rate of coronary blood in the absence of any significant changes in heart rate or the ECG. The increased coronary blood flow lasted for a period of 20 to 30 minutes. RA-8 was quite effective in counteracting the coronary vasoconstriction induced by the administration of pitressin. This effect too occurred in the absence of any significant changes in heart rate, b.p. or the ECG. (Supported by Grant #H2354C2 from the Dept. of Health Education and Welfare, USPHS)

16 *Pharmacology*

EFFECT OF QUINIDINE ON THE ELECTROLYTE CONCENTRATIONS OF THE DOG HEART. Victor Feldman* and Theodore R. Sherrod. Dept. of Pharmacology, Univ. of Ill. Coll. of Med., Chicago, Illinois.

It is now established that digitalis causes an intracellular depletion of K in cardiac muscle, and that K is pharmacologically antagonistic to the action of digitalis. A similar relationship between the action of quinidine and digitalis has never been adequately demonstrated. The purpose of this investigation was to determine quantitatively the effects of quinidine on the K content of heart muscle. Twelve adult male dogs weighing approximately 20 kilograms each were used in this experiment. Six dogs were infused intravenously with an average of 250 cc. of 3.0 mg./cc. quinidine sulphate solution in .9% NaCl. The drug was administered until there was a significant widening of the QRS complex. Three blood samples were drawn at 15-minute intervals from each dog for plasma sodium and potassium levels. The control animals were infused with .9% NaCl. The heart was then removed, immersed in distilled H_2O, and rinsed free of blood. A 2-gm. sample of ventricular and auricular muscle was obtained from each heart. By dry ashing and diluting the residue to appropriate volumes it was possible to determine the Na and K concentrations spectrophotometrically. In each case there was a significant rise in the intracellular K concentration in those animals receiving quinidine, as compared to the control group. There was no significant change in intracellular Na. (Supported by Grant H2354C2, Dept. of Health Education and Welfare, USPHS.)

17 *Pharmacology*

GUANETHIDINE IN THE TREATMENT OF DIASTOLIC HYPERTENSION. Albert N. Brest* and John H. Moyer. Dept. of Medicine, Hahnemann Medical College and Hospital, Philadelphia, Pa.

Guanethidine is a new antihypertensive drug with a hypotensive potency similar to that of the ganglionic blocking compounds. It has a rather unique mechanism of action which consists of an inhibition of the peripheral release of catecholamines from the postganglionic sympathetic fibers. The blood pressure reduction obtained is predominantly a postural one. However, in contrast with the gangliopleglic drugs, guanethidine produces a significant hypotensive response without the associated side effects due to parasympathetic blockade. The dosage required to obtain a significant response varies from 25 to 450 mg per day, with an average dosage of 100-150 mg daily. Blood pressure reduction may not occur for 24 to 48 hours following the onset of therapy, but may subsequently persist for 4 or more days after the drug is stopped. The antihypertensive effectiveness of this compound is further enhanced by the concomitant administration of a thiazide derivative. This new antihypertensive agent appears to hold particular promise for those hypertensive patients with the more severe degrees of diastolic blood pressure elevation.

18 *Nutrition*

MAGNESIUM DEFICIENCY IN DUCKS. J. Di Giorgio*, J. J. Vitale and E. E. Hellerstein. Dept. of Nutrition, Harvard School of Public Health, and Dept. of Pathology, Harvard Medical School, Boston, Mass.

Feeding a magnesium-deficient diet to ducklings resulted in lowered serum cholesterol and protein levels as well as lowered QO_2's for heart mitochondria. Incubation of mitochondria from control and deficient ducks in a magnesium-free media resulted in decreased QO_2's although this effect was more marked in deficient animals. P/O ratios for heart mitochondria from magnesium-deficient or control ducks were the same. However, no phosphorylation occurred by mitochondria from deficient ducks when incubated in a magnesium-free media. Magnesium deficiency produced no apparent effect on ATPase activity, nor on the effect of dinitrophenol thereon, of heart mitochondria. The results seem to indicate that mitochondria isolated from deficient ducks lack only magnesium, an activator, but not the enzymes for oxidation and phosphorylation. Ducks maintained on a magnesium-deficient diet showed moderate calcium deposition in the kidneys and portions of the myocardium. These changes were encountered less frequently in the high dietary magnesium animals.

19 *Immunology*

HIGH SPEED CINEPHOTOMICROGRAPHY OF HEMODYNAMICS. E. H. Bloch, Western Reserve U. (Motion Picture).

The purpose was to define the flow dynamics of blood cells in living circulation. Blood flow in normal animals was recorded from: mesentery, liver (frog), lung (rabbit) at objective mgf X10, 22, 44, 50 in white light and at 576, 549, 435, 407 mu (frog mesentery), at 480 to 3720 frames per sec. (fps) on color and monochromatic film. The vessels ranged from 500 to 9 u in diam. The film illustrates the principles in frog mesentery. What appears as a homogeneous laminated column of cells to the eye as seen through the microscope or on film at 8-64 fps is not so when recorded at 3000+fps and analyzed at 16fps. Flow is turbulent. Cells are randomely oriented, often at right angle to the direction of flow. They describe a complex helical pattern, frequently rotating about their short axis and moving across cell "columns" in each segment of vessel (between branches). At bifurcations further reorientation occurs. Cellular concentration, unit vol/unit time (1 msec) is most variable in arterioles. The peripheral plasma layer is variable, frequently not discernable. Cellular reorientation is greater in the arterial than in the venous system.

XUM

054. CARDIOVASCULAR SYSTEM–Blood Flow

1 *Physiology*

BLOOD FLOW DISTRIBUTION IN INTACT DOGS BY ULTRASONIC FLOWMETERS Dean Franklin, Don Baker, Robert Van Citters and Robert F. Rushmer (intr. by J. W. Stutzman.) Dept. of Physiology & Biophysics, Univ. of Washington School of Med., Seattle, Wash.

The mechanisms of peripheral vascular control must be described ultimately in terms of their participation in partitioning volume flow during spontaneous activity by intact individuals. To study the time course and extent of changes in flow distribution, instantaneous flows through various vascular beds in intact dogs were registered continuously with pulsed ultrasonic flowmeters. The flow patterns in the root of the aorta, abdominal aorta, renal artery, iliac artery, hepatic portal vein and inferior vena cava were quite distinctive. For example, the wave form of the flow pulse in the renal artery had an abrupt ascending limb with a sustained systolic plateau and a gradual run-off, differing materially from the pattern in the adjacent abdominal aorta. To record flow through two or three vascular beds simultaneously required a time-sharing technique to preclude interaction between the flowmeters. Flow was sampled from each flowmeter individually in rapid succession (400 c.p.s.) without interfering with adequate frequncy response of the gauges. The flow through various vascular beds was unexpectedly uniform during spontaneous exercise, including running on a treadmill at 3 m.p.h. on a 5% grade. (Supported by a grant from the American Heart Association.)

2 *Physiology*

GRADIENT OF BLOOD FLOW IN RAT SMALL INTESTINE. Sheldon H. Steiner and Gustave C. E. Mueller (intr. by L.A. Sapirstein). Acceleration Section, Biophysics Branch, Aerospace Medical Lab. WADD, W-P AFB, Ohio.

The generally held belief that there is a gradient in blood flow through the small intestine rests primarily on anatomical evidence. The indicator fractionation technique employing Rb^{86} was used to measure the distribution of blood flow in the small intestine of fasted intact rats anesthetized with sodium pentobarbital. The method depends on the fact that during the first minute intravenously injected Rb^{86} is almost completely extracted from the blood by those tissues in which the potassium is rapidly exchangeable with plasma potassium; it is therefore distributed among tissues in the same way as blood flow. In 20 rats injected intravenously with Rb^{86} and killed within one minute the small bowel was stretched evenly using a 20 gm weight and divided lengthwise. Rb^{86} uptake per unit length showed a distinct and regular gradient from duodenum (12.5-7.0% small intestinal Rb^{86}/inch) to ileum (4.8-3.8%/inch). The gradient is a little less striking when expressed on a weight basis but is still quite evident. (The views expressed are those of the authors and are not to be construed as representing official Air Force policy.)

3 *Physiology*

DISTRIBUTION OF BLOOD FLOW TO THE HUMAN EXTREMITY IN RESPONSE TO PHYSIOLOGIC AND PHARMACOLOGIC STIMULI. Walter Redisch, Italo Besseghini and J. Murray Steele. NYU Research Service, Goldwater Memorial Hospital and NYU College of Medicine.

Differential plethysmography was used to ascertain the respective participation of skin and muscle vascular beds in the lower extremity of man in responses to stimuli. Reflex responses to warming and cooling and direct response to exercise were used as physiologic stimuli. The skin participated much more than the muscles in blood flow increments and decrements, achieved by body warming and cooling. In contrast, muscle flow received by far the greater share from increments in response to exercise. Known vasoactive substances were used as pharmacologic stimuli. Nor-adrenaline reduced muscle flow much more than skin flow. Adrenalin markedly reduced skin flow and slightly increased muscle flow. Blood flow increments in response to adrenergic and postganglionic blocking agents went predominantly to the skin. In contrast, most of the increment following nylidrine, isoxsuprine and nicotinic acid went to the muscles. The increase in blood flow following amino-alcohol was distributed equally between skin and muscles.

4 *Physiology*

THEORETICAL CONSIDERATIONS CONCERNING "HEART-LUNG BYPASS" PROCEDURES. Pierre-M. Galletti* and Gerhard A. Brecher. Dept. of Physiology, Emory University, Atlanta, Ga.

"Heart-lung bypass" is a technique for maintaining, with the help of mechanical pumps and gas exchange devices, life-sustaining hemodynamic and metabolic conditions in the absence of cardiac and pulmonary functions. Although intended to mimic normal circulation, extracorporeal circulation differs in several respects: 1) the direction of flow in the venous and arterial tree is partially reversed, 2) the distribution of blood among different organs is altered, 3) laminar patterns of flow in the extracorporeal circuit are seldom achieved, 4) high flow velocities commonly occur across cannulae, 5) the blood is infused into the arterial tree under a variety of pulsatile conditions, 6) the blood behaves as a random mixture of corpuscles in plasma, 7) neuroregulatory mechanisms may be deprived of their effective organs, 8) the blood can be equilibrated in the artificial lung with any gas mixture in any desired amount. It is concluded that in the development of heart-lung bypass procedures, as much attention should be devoted to the perfused organism as to the mechanical devices. Then extracorporeal circulation offers a valuable tool for the study of organ functions under unusual conditions.

5 *Pharmacology*

EFFECT OF THIODRIDAZINE ON RAT LIVER HEMODYNAMICS. G.L. Plaa, G.J. Blacker, E. C. McGough and J.M. Fujimoto (intr. by F. W. Schueler.) Dept. of Pharmacology, Tulane University School of Medicine, New Orleans, Louisiana.

Thioridazine (Mellaril, TP-21), when perfused in recirculating albumin-Locke's solution through isolated rat livers produces the following: 1) a decrease in perfusate flow through the organ, 2) an increase in portal pressure, and 3) decrease in organ weight. These effects were consistently demonstrated with doses of 4 mg (initial perfusate concentration = 25 μg/ml) but were quantitatively more pronounced and of longer duration with higher doses. With 20 mg the perfusate flow decreased from a normal value of 6 ml/gm/min to a value of 1.5 ml/gm/min; simultaneously the portal pressure increased 30-40 mm (H_2O); the organ weight decreased by 4 units (arbitrary scale). Values returned to normal within 30 min. Tachyphylaxis did not occur following several administrations of 4 mg thioridazine. Sodium nitrite (10 mg) prevented the decrease in perfusate flow caused by 6 to 10 mg thioridazine, but did not attenuate the rise in portal pressure or the decrease in organ weight. (Supported by grants from Sandoz Pharmaceuticals and Louisiana Heart Association.)

055. CARDIOVASCULAR SYSTEM–Blood Flow–Coronary

1 *Physiology*

CORONARY BLOOD FLOW USING RADIOACTIVE IODINE COMPARED WITH NITROUS OXIDE. **Ivan Forte,* A. Jane Williams,* J. E. Schmitthenner,* H. Neal,* H. Woske,* R. Richards,* and J. H. Hafkenschiel.** **Lankenau Hospital, Philadelphia, Pa.**

Attempting to explore other methods that might be capable of measuring coronary blood flow in man with minimal trauma, in DUPM anesthetized dogs simultaneous measurements of coronary blood flow and cardiac output were compared with calculations made from isotope dilution curves of blood drawn through catheters from coronary sinus, proximal pulmonary artery and femoral artery in fifteen experiments. Known quantities of isotope were injected as a bolus and immediate radiodilution curves on a two channel "Rectiriter" were obtained by withdrawal of blood through a glass helix in a scintillation counter. An external monitor focused on the heart area recorded directly with one of the other three sites. Labeled coronary circulated blood emptying into the coronary sinus could not be separated from blood returning to the pulmonary artery from other vascular regions. No pattern of relationship could be established between nitrous oxide coronary flow measurements and values calculated from the few satisfactory dilution curves. The curves recorded simultaneously from the precordium with the coronary sinus blood suggest no way of approximating coronary blood flow. We conclude, as have others, that this method is impracticable in the dog. Supported by grant H1817C5 from P.H.S.

2 **Physiology**

The Effect of Experimental Aortic Stenosis on Coronary Circulation. Marvin R. Blumenthal*, Hsueh-Hwa Wang* and S. C. Wang. Department of Pharmacology, College of Physicians and Surgeons, Columbia University, New York City.

In dogs anesthetized with morphine and chloralose, acute aortic stenosis was produced by a special devise with an adjustable aperture. The coronary sinus outflow and the left ventricular output were measured with rotameters; and the arterial blood pressure and left intraventricular pressure by pressure transducers. Arterial and serial coronary sinus blood O_2 contents were also determined. The presence of aortic stenosis was ascertained by the increase in left intraventricular pressure and the decrease in pulse pressure. The coronary sinus outflow almost invariably increased, usually accompanied by an increase in coronary A-V O_2 difference. Mean arterial blood pressure and left ventricular output usually decreased. The changes in coronary circulation could best be explained by vasodilatation of the coronary bed, since it occurred despite a decrease in perfusion pressure and a probable increase in extravascular support. Aortic constriction with comparable changes in left intraventricular pressure and left ventricular output was also produced. With a higher coronary perfusion pressure a greater increase in coronary sinus outflow was noted without an increase in coronary A-V O_2 difference. (Supported by a grant from USPHS Grant B31C.)

3 **Physiology**

EFFECTS OF NICOTINE AND CIGARETTE SMOKE ON THE DOG BALLISTOCARDIOGRAM. Aldo N. Corbascio and J. W. West (intr. by H. I. Kohn.) Depts. of Pharmacology and Therapeutic Research, University of Pennsylvania Medical School, Philadelphia, Pa.

Nicotine (0.2 μg/kg) was selectively injected into the anterior descending branch of the left coronary artery of intact-chest dogs by means of a rigid catheter, introduced fluoroscopically. The dogs, which were lightly anesthetized with morphine (3 mg/kg IM) and a mixture of Dial-urethane and Nembutal IV (0.25 ml/kg), were placed on an ultra low frequency ballistocardiograph, and BCG, ECG, right atrial and femoral arterial pressures were recorded concurrently. Respiration was stopped by the administration of decamethonium bromide (0.2 mg/kg IV) while an adequate tidal volume was maintained by a respiratory pump. The intracoronary administration of nicotine or its congener (DMPP) was followed by a marked improvement of the ballistic tracing while heart rate, ECG, and systemic arterial pressure remained unchanged. The IV administration of nicotine (5 μg/kg) produced a diphasic response characterized by an initial systemic arterial pressure increment which induced deterioration on the BCG tracing, followed by a normotensive phase with a concomitant improvement in the BCG tracing. This picture could be faithfully reproduced by inducing the animals to inhale cigarette smoke through the intake of the respiratory pump.

4 **Physiology**

REACTIVE HYPEREMIA FLOW CHARACTERISTICS OF CANINE MYOCARDIUM. Jay D. Coffman* and Donald E. Gregg. Walter Reed Army Inst. of Research Wash. 12, D. C.

Blood flow before and after occlusion of cannulated circumflex arteries of 9 anesthetized open chest dogs was studied using a constantly recording rotameter. Following occlusion, flow in excess of control flow (R.H.) and duration of R.H. increased proportionally with length of occlusion (5 to 180 sec.) Peak flow during R.H. often reached a height with a certain duration of anoxia beyond which it did not rise with longer periods of occlusion. Flow debts estimated from control flows before occlusion were always overpaid (averaged 208% of control flow). Heart rates remained stable while blood pressures dropped during anoxia and returned during R.H. In 8 of these dogs, 3 to 7-30 sec. occlusions were successively performed. R.H. flows (mean 21.4 ± S.D. 2.3 cc.), peak flows (77 ± 2.5 cc.) and durations (1.4 ± 0.5 min.) were consistent in the same dog. In 15 dogs, 2 R.H. controls were compared to R.H. performed during drug infusion into the circumflex artery. Effects were variable; epinephrine, levarterenol and isoproterenol often increased while pitressin decreased R.H. flow. Conclusions are: myocardial R.H. flow varies proportionally with duration of anoxia, estimated flow debt for myocardial anoxia is overpaid and the drugs tested have a variable effect on R.H.

5 **Physiology**

THE EFFECTS OF ISCHEMIA AND OPERATIVE ATTEMPTS AT MYOCARDIAL REVASCULARIZATION ON THE VENTRICULAR FIBRILLATION THRESHOLD. C. M. Phibbs*, R. A. vanTyn*, and L. D. Maclean. Dept of Surgery, Univ. of Minn. and Ancker Hosp., St. Paul.

In 76 dogs the ventricular fibrillation threshold was determined and one of the following performed: 1) Cardiac poudrage, 2) Internal mammary artery implantation into the myocardium, 3) Pulmonary artery to left atrial shunt, 4) Permanent ligation of the left anterior descending coronary artery, 5) Thoracotomy only. In one-half of groups 1, 2 and 5 chronic mild ischemia was produced by ligating one small branch of each of the anterior descending and circumflex arteries. The ventricular fibrillation threshold was redetermined with and without acute coronary occlusion 5 to 6 months after the original procedure. Chronic mild or massive ischemia, pulmonary artery to left atrial shunt and thoracotomy alone had no effect on the ventricular fibrillation threshold. The Beck operation and internal mammary artery implantation produced a significant elevation of the threshold with coronary occlusion. The latter was significantly better than the former. The results suggest that a greater resistance to ventricular fibrillation due to coronary occlusion can be achieved by operative means than by either mild or severe ischemia alone.

6 **Physiology**

THE EFFECT OF NITROGLYCERINE ON CORONARY VASCULAR RESISTANCE D. M. Griggs, Jr., R. N. Pierson, Jr., and R. B. Case (intr.by L. B. Dotti.) Dept. of Med., St. Luke's Hosp., New York, N. Y.

The effect of nitroglycerine on coronary vascular resistance (CVR) was studied in the dog by fixing coronary flow and noting as the response a drop in coronary perfusion pressure. Blood was delivered by an occlusive pump from a femoral artery through a rotameter to the left main coronary artery via a Gregg cannula. Continuous recordings were made of coronary flow, right atrial pressure, aortic pressure, and mean perfusion pressure just proximal to the coronary cannula. CVR was expressed as pressure gradient/flow. A one ml solution of nitroglycerine in saline or blood was injected into the perfusion circuit. A control sample without nitroglycerine was also injected. Delivery time from the site of injection to the coronary artery was known for the flow rate so that the time of onset of response could be related to the presence of the agent. The results demonstrated a prompt fall in CVR when the drug reached the coronary circulation before a change in heart rate or aortic pressure occurred. The percentage decrease in CVR was related to the dose administered as well as to the initial CVR. It was concluded that nitroglycerine produces a prompt, dose related reduction in CVR.
(Supported by the New York Heart Assn., and the U. S. Public Health Service.)

056. CARDIOVASCULAR SYSTEM–Blood Flow–Peripheral

1 **Physiology**

PRESSURE VOLUME RELATIONSHIPS OF SMALL ARTERIAL SEGMENTS. P.C. Johnson, W. L. Knochel*and T. L. Sherman* Dept. of Physiol. Ind. Univ. Med. School. Indianapolis, Ind.

Pressure-flow studies in several organs have indicated an inverse relation between arterial pressure and resistance vessel diameter best explained as a myogenic response of the vessel wall. However, pressure-volume studies of arterial segments in vitro have uniformly shown that pressure elevation increases vessel diameter. The present study investigated the pressure-volume relationships of small arterial segments (0.4 to 3.0 mm. i.d.) in situ under conditions comparable to the pressure flow studies. Arterial segments of the forelimb and hind limb of pentobarbitalized dogs were freed from surrounding tissue and a side branch catheterized to communicate with volumetric tubing. Clamping for 30 sec. without changing pressure usually did not signifiaantly change volume. However, if the vessel was clamped and internal pressure elevated, the volume first increased, then decreased, often to less than control values in 30 sec. Vessel size was related to response; 12 of 16 segments below 1.2 mm. i.d. actively responded while all 4 above this size were passive. Dilation with pressure reduction was also observed, but was less frequent. The results support the myogenic hypothesis. (Supported by Indiana Heart Association.)

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2 **Physiology**

GENUINE AUTOREGULATION OF THE RENAL CIRCULATION. R. G. Shanks* and W. H. Waugh. Dept. of Physiol., Med. Coll. of Ga., Augusta.

We present further evidence that renal autoregulation is due to an active vasoconstrictive change and not to changes in tissue pressure. Isolated dog kidneys were perfused with blood directly from a donor dog or with a colloid (20% plasma - 80% PVP-Locke solution) from a pressurized reservoir. Continuous records of renal flow, arterial pressure and deep venous pressure were obtained. A sudden (1 sec.) increase in arterial pressure produced an increase in renal flow and deep venous pressure, maximal in about 2 sec.; both declined over the next 4-6 sec. and flow reached a steady state to show autoregulation; however, deep venous pressure rose gradually during maintained high arterial pressure (45-120 sec.) to about the level of the initial peak. When flow became steady the increased resistance to flow was almost all in the "pre-venous" vessels. Chloral hydrate (0.5 Gm.%) in the perfusate abolished the rapid decline in flow which normally followed the peak flow; thus no genuine autoregulation occurred. However there were much greater increases in tissue pressure which at times caused a factitious autoregulation. Cooling of the kidney to 3-10°C. gave results like those with chloral hydrate.

Supported by P.H.S. Senior Research Fellowship SF-243 (Dr. Waugh) & by a Cardiovascular Research Training Program (Dr. Shanks) through P.H.S. grant HTS-5044 and the Am. Heart Assoc.

3 **Physiology**

ADAPTATION OF THE ELECTRICAL IMPEDANCE METHOD TO THE STUDY OF CIRCULATION IN THE TOOTH PULP. F. Liebman and F. Cosenza (intr. by C. W. Hampel.) N.Y.U. Dental Coll. and Murry and Leonie Guggenheim Foundation Inst. for Dent. Res.

In vivo study of circulation in the tooth pulp has long been a goal of investigators in the field of oral physiology. The electrical impedance method has been adapted to this study in dogs. The canine teeth were used because of location and anatomy. Tetrapolar, cast gold electrodes were placed in four cavities prepared on the labial side of the tooth. The dentin layer overlying the pulp in the cavity preparation should not exceed 250μ. Electrode jelly was used to improve conductivity. Simultaneous recordings of the EKG and pulp impedance changes were made. Since the pulp is enclosed in a rigid-walled chamber, the pulsatile variations in impedance cannot result from changes in blood volume of the pulp, but are explained as the result of changes in RBC distribution related to blood velocity changes in the vessels during the cardiac cycle. The above method permits the study of circulation in the intact tooth pulp, precise relocation of electrodes over the pulp in successive experiments, and graphic recordings from this relatively inaccessible part of the circulation. (We wish to thank Mr. Sam Bagno of Walter Kidde and Co. Inc., for designing the electronic circuits.)

4 **Physiology**

CHANGES OF EXTRACRANIAL CIRCULATION DURING HYPOXIA IN YOUNG AND OLDER HEALTHY MEN. Ernst Simonson. Laboratory of Physiological Hygiene, University of Minnesota, Minneapolis, Minn.

Impedance plethysmographic pulse tracings from the forehead were obtained before and in the 10th min. of breathing a 10% O_2, 90% N_2 mixture. Amplitude and interval changes were compared in 42 young healthy men (18 to 30 years) and 58 older men (55 to 65 years) with a drop of the arterial O_2 saturation (recorded through an ear lobe oximeter) exceeding 10 per cent. There was no significant change of the peak amplitude and height of the dicrotic notch in the young men during hypoxia, in contrast to a highly significant increase in the older men ($P < .001$, t-test). In both groups there was a statistically highly significant acceleration of the heart rate and lengthening of the relative peak time ($\frac{\text{Peak time}}{\text{cycle}} \times 100$), which was more pronounced in the older men. The greater changes of extracranial circulation in the older men during hypoxia, particularly in the amplitudes may be interpreted as a loss of stability in peripheral circulation during stress, similarly to previous observations on circulatory postural adjustment. (Supported by U. S. P. H. S. grant B 1859.)

5 **Physiology**

POLAROGRAPHIC EVALUATION OF THE EFFECTS OF VASCULAR TRAUMA AND DRUGS ON MUSCLE OXYGEN TENSION. S.I. Schwartz, P.D. Harris and E.B. Mahoney (intr. by W.T. Pommerenke) Univ. of Rochester School of Med. and Dent., Rochester, N.Y.

Measurement of oxygen available at the cellular level has been used to reflect changes in arterial circulation. By means of platinum electrode polarography, tissue oxygen tensions in the gastrocnemius muscle were determined: (a) during arterial occlusion; (b) following trauma to the major artery of control and sympathectomized limbs; and (c) following trauma to the artery after creating a vascular pedicle. Effects of intra-adventitially administered procaine were compared with intra-arterial priscoline and papaverine.

An abrupt and significant reduction in the oxygen tension of muscle occurred when a major vessel was dissected clear from surrounding tissues and also when the vessel was occluded. This was greatest in the vascular pedicle preparations and least in sympathectomized limbs. Reduction in muscle oxygen tension resulting from dissection and occlusion of a major vessel other than the one supplying the muscle was also demonstrated. Intra-arterial papaverine was most effective in counteracting the post-traumatic reduction in muscle oxygen tension. The effects of procaine and priscoline were less marked.

6 **Physiology**

CARDIOVASCULAR EFFECTS OF DISTORTION OF STRETCH RECEPTORS IN THE CARDIAC WALLS. F. W. Klussmann, R. L. Van Citters and R. F. Rushmer (intr. by Rudolf Thauer.) Dept. of Physiology & Biophysics, Univ. of Washington School of Med., Seattle, Wash.

Stretch receptors within the walls of the heart chambers and central veins have been described by many investigators, but little is known about the effects of these receptors upon the cardiovascular system itself. In dogs different parts of the cardiac walls were selectively distorted by means of suction cups, and distortion of the great veins was achieved with an umbrella-like instrument. Arterial pressure was measured in the femoral artery with a Statham strain gauge (P23A) and recorded with a Sanborn Polyviso. Changes in the diameter of the liver and in the circumference of the upper hind leg were recorded in terms of changes in resistance of a mercury column within a length of thin rubber tubing. Distortion of the different parts of the heart walls and the great veins generally produced premature ventricular contractions with no changes in systolic or diastolic arterial pressure or in the heart rate. The measured dimensions of the liver and the hind leg showed no consistent changes. In one experiment suction applied to the right atrium or to either ventricle produced a drop in systolic and diastolic arterial pressure. (Supported by Cardiovascular Training Grant HTS 5147 from the National Heart Institute.)

7 **Physiology**

VASCULAR RESPONSES TO ELECTRICAL STIMULATION OF THE SYMPATHETIC TRUNK IN THE DOG. R. O. Rawson and W. C. Randall, Dep't. of Physiology, Stritch School of Med. and Grad. School, Loyola Univ., Chicago, Ill.

Using miniature photoelectric plethysmographs, vasomotor responses have been recorded simultaneously from 3 cutaneous and 1 muscle bed in the hind limb of the dog. Patterns of preganglionic vasomotor pathways to the limb were mapped by electrical stimulation at each interganglionic segment of the lumbar sympathetic trunk. In most animals, pathways innervating the thigh, calf and footpad skin enter at similar levels between the third and fifth vertebral levels. In some animals, however, entirely separate levels of entry for each area exist between L2 and L7. With notable exceptions, vasoconstriction became more intense in all skin areas as the electrodes were successively moved caudalward. Cutaneous pulses were generally larger and constrictor responses more intense in pad skin than in thigh and calf skin. The gastrocnemius muscle is supplied with vasomotor fibers, but neither constriction nor dilatation reached intense proportions in response to direct electrical stimulation of the trunk.

8 **Physiology**

DIFFERENTIAL ALTERATIONS IN MESENTERIC ARTERIAL AND VENOUS RESISTANCE IN HEMORRHAGE SHOCK. J.J. FRIEDMAN. DEPT. OF PHYSIOLOGY, INDIANA UNIV. MED. CENTER, INDIANAPOLIS.

Differential alterations in mesenteric arterial and venous resistance during the development of hemorrhagic shock in dogs were inferred from differential changes in mesenteric plasma volume and vascular resistance. Mesenteric plasma volume was estimated from the portal venous dilution curves of radioiodoalbumin injected into the superior mesenteric artery. Mesenteric vascular resistance was calculated from the mesenteric artery-portal vein pressure gradient and mesenteric flow. Hemorrhage was carried out by means of a modified Lamson bottle technique. Following early hemorrhage resistance remained unchanged while volume declined. During the phase of autoreinfusion resistance proceeded to rise and volume declined further. With reinfusion resistance declined and volume increased in a transient fashion. During the terminal phase of shock, resistance was markedly elevated and volume reduced. Indications of differential arterial and venous activity were observed throughout the experiment as changes in resistance without the associated change in volume. These discrepancies were attributed to changes in venous outflow. It would appear that arterial resistance influences tissue blood flow and venous resistance determines tissue blood volume.

9 **Physiology**

THE MECHANISM OF EDEMA PRODUCTION BY ACETYLCHOLINE, SEROTONIN, HISTAMINE AND NOREPINEPHRINE. F. J. Haddy, VA Res. Hosp. and Northwestern Univ. School of Med., Chicago.

Small venous pressure in the dog paw may rise during infusion of the above agents into the brachial artery (Minn. Med. 41:162, 1958). The pressure rise results from arteriolar dilation, venous constriction, arteriolar dilation and venous constriction, and venous constriction out of proportion to arteriolar constriction, respectively. These findings suggested that each agent might raise capillary hydrostatic pressure and produce edema. Hence, forelimb weight and small venous pressure were measured during prolonged infusion of each agent into the brachial artery of 33 denervated forelimbs. Infusion rates were maintained below those having significant effects upon brachial arterial pressure. During infusion of saline, small venous pressure and weight gain averaged 10 mm. Hg. and 0.1 grams/min. Corresponding values during infusion of acetyl-beta-methylcholine, serotonin and histamine were 13 and 0.3, 16 and 0.4, and 18 and 1.2. Norepinephrine increased small venous pressure in 8 of 19 experiments to levels as high as 44 mm.Hg. These limbs gained weight. In the remaining instances small venous pressure and weight did not change. These findings indicate that each of these agents can produce edema by raising capillary hydrostatic pressure.

10 **Physiology**

EFFECTS OF ANOXIA ON VASCULAR RESISTANCE OF HINDLIMB. A. Dil, J. Litwin and D. M. Aviado (intr. by Carl F. Schmidt). Dept. Pharmacology, Univ. Penna. Schools of Med., Phila., Penna.

Perfusion of hindlimb of anesthetized dog shows two types of responses: vasoconstriction during inhalation of 5% oxygen and vasodilatation during reoxygenation. Both responses are eliminated by transection of cervical spinal cord. Denervation of chemoreceptors or combined adrenalectomy and blockade of sympathetic nerves by bretylium reduces the vasoconstrictor but not the vasodilator response. The vasodilatation during reoxygenation arises from excitation of intracranial centers. The responsible vasodilator nerves are partially blocked by either hexamethonium or atropine but are resistant to sympathetic blocking drugs.

11 **Physiology**

INDICATOR CONCENTRATION CURVES IN DOGS FOREPAW. George Lin*, Carlos Rapela*, Adam B. Denison, Jr. and Harold D. Green. Bowman Gray School of Medicine, Winston-Salem, N. C.

Use of indicator concentration curves in calculating blood flow and vascular volume was evaluated by recording arterial inflow into the isolated dog's forepaw while injecting 0.25 mg of Cardiogreen during 1 sec into a mixer in the arterial supply and recording the dye concentration by sampling from a mixer in the vein draining the paw. In 10 experiments metered flows averaged 20.8 (range 4.4 to 44.2) ml/min. The calculated flows (dye) averaged 134.0% (range 65 to 356) of the metered flow. The mean transit times averaged 25.1 (range 7.6 to 59.3) sec. The vascular volumes averaged 8.5 (range 2.5 to 25.2)ml/paw. The last volumes of four experiments averaged 93.5 (range 58 to 123) % of that computed from blood hemoglobin concentration and the volume and Hb concentration of the saline "wash out" of the paw. The flows, and vascular volumes increased and the transit times decreased with increase of perfusion pressure. Conclusion: with present technic the indicator concentration technic gives a good "order of magnitude" measure of the desired parameters. Supported by grants H-487, HTS 5392, National Institutes of Health.

12 **Physiology**

EVALUATION OF VENOUS OCCLUSION PLETHYSMOGRAPHY
Margaret C. Conrad*, Adam B. Denison, Jr., and Harold D. Green
Bowman Gray School of Medicine, Winston-Salem, N.C.

In order to evaluate the venous occlusion method for blood flow measurement, inflow and outflow of the amputated dog's paw were measured with electromagnetic flowmeters simultaneously with plethysmographic flow. Venous tamponade was complete for a period of time which was dependent on the height of the occlusion pressure and the magnitude of arterial flow. Paw venous pressure rose rapidly to "effective occlusion pressure", at which time blood began to escape beneath the occlusion cuff. Arterial flow, which began declining almost immediately after venous occlusion, averaged - 9% at 4/5 sec. Two occlusion artifacts were observed. The first, an abrupt deflection, is readily avoided in measuring the slope of the plethysmographic record. The second, a slower rise, can be detected only when the arterial inflow is interrupted prior to inflation of the cuff. The plethysmographic volume showed the customary decelerating rise due to progressively diminishing inflow and acelerating outflow. The best fit of the plethysmographically measured flow to the preocclusion flow was obtained during the 3/5 sec. interval beginning 2/5 sec. after venous occlusion. The arterial inflow averaged 98.9% of the plethysmograph flow with a mean deviation of ± 11%. (Supported by Grants H-487 and HTS 5392, Nat. Insts. of Health)

13 **Physiology**

INDUCED CHANGES IN THE CAPACITANCE OF THE TURTLE VENOUS SYSTEM. Arthur H. L. From (Intr. by H. Rostorfer) Anatomy & Physiology Dept. Indiana U. Bloomington.

Injection of epinephrine and nor-epinephrine into the circulation induced a state characterized by a slowly beating, greatly dilated heart and tensely dilated great vessels. Arterial blood pressure, closing pressure, and intraventricular pressure were measured on pithed and unpithed turtles before and after epinephrine injection. Proximal and distal ends of a systemic aorta were connected to a reservoir and changes in reservoir volume measured, after injection of epinephrine and nor-epinephrine and, in another series, after occlusion of all central arteries but the one leading to the reservoir. Arterial blood pressure and closing pressure were greatly elevated in treated and physiologically excited animals. The latter failed to respond to injected epinephrine. Intraventricular systolic and end-diastolic pressures were elevated in injected animals, but only systolic pressure increased significantly in animals with truncus occluded. In the epinephrine series reservoir volume increase was three times that of the occlusion series. It was concluded: 1. Both physiological and epinephrine induced states represented an increased vascular tone; 2. The turtle venous system is capable of markedly decreasing its capacitance in response to endogenous sympathetic activity of sympathomimetic drugs.

XUM

14 **Physiology**

BLOOD FLOW CHANGES IN EXTREMITIES OF DOGS FOLLOWING SYMPATHECTOMY AND EXERCISE. H.E.Ederstrom and D.A.Porter* Dept.Physiol. & Pharm., U.North Dakota Med.Sch., Grand Forks, N.Dak.

Unilateral lumbar sympathectomy was performed on dogs and recovery permitted. Control blood flows in the operated leg were about 40% below the normal leg. Dibenzyline, 2.5 mg/kg, did not alter this difference. Hind-legs were exercised by direct muscle or sciatic nerve stimulation doubling blood flow almost immediately but the difference between normal and sympathectomized legs persisted. Without Dibenzyline, exercise failed to increase blood flow in the normal leg during stimulation, but the usual increase occurred in the operated leg, indicating that sympathetic vasomotor control had been removed. Conclusions are: sympathectomized vessels remain in a fixed state of tone while normal vessels relax under deep anesthesia; exercise increases blood flow almost immediately due to the massaging action of the muscles; local metabolites caused the post-exercise hyperemia; circulating metabolites were not involved in this hyperemia since exercise of the forelegs did not increase blood flow in the quiescent hind-legs; and the observation that the combined effects of Dibenzyline and exercise failed to dilate the denervated to the level of the normal vessels suggests that the sympathectomized vasculature has undergone an anatomical rather than physiological or sensitivity change. (Supported by Life Ins. Research Fund grant).

15 **Physiology**

IN-VIVO ESTIMATION OF REGIONAL COLON BLOOD FLOW. Marvin Bacaner and Myron Pollycove. (intr. by John H. Lawrence.) Donner Lab., Univ. of Calif., Berkeley, California.

Lack of an in vivo method for measuring colon blood flow has limited the study of the functional physiology and pathology of the colon relative to this critical parameter. A technique has been devised in which a specially designed Geiger-Muller probe (passed per rectum into the colon) measures the dilution curve of a bolus of intravenously injected P-32 chromic phosphate. The short range of the beta particle virtually restricts the monitored field to the surrounding colon. Simultaneous cardiac output and liver blood flow measurements are made to aid interpretation of the colon curve. The derived curve represents several aspects of colon circulation: a)circulation and transit times, b)fraction of cardiac output perfusing the monitored area, c)volume flow, d)magnitude of existing blood pool, e)regional turnover of blood. Circulatory patterns (curve shapes) have been characterized for the dog colon under normal conditions and during procedures which decrease and increase circulation (vascular occlusion, hemorrhage, saline infusion, autonomic stimulation and mediator drugs). This technique appears useful in studying a)functional physiology, b)vascular diseases and c)drug effects. Human studies are currently in progress.

16 **Physiology**

EFFECT OF PARTIAL VENOUS OCCLUSION AND RELEASE ON MUSCLE OXYGEN UPTAKE AND BLOOD FLOW. J. T. Fales, S. R. Heisey and K. L. Zierler (intr. by Janet Howell Clark.) Johns Hopkins Univ. Schools of Hygiene and Medicine, Baltimore, Md.

It was reported previously that stretch of the dog gastrocnemius-plantaris muscle, in vivo, decreased muscle O_2 uptake. There was little or no repayment of "O_2 debt" on release of stretch. A possible explanation of this effect may be that muscle stretch occludes venules. Accordingly the effect of partial venous occlusion was studied. During partial venous occlusion blood flow and muscle O_2 uptake decrease. On release, both return to or exceed control values. "O_2 debt" is either not repaid or it is repaid over a relatively long time in contrast to the familiar effect of arterial occlusion. (Performed under contract with ONR and supported by Grant A-999 from NIAMD.)

17 **Physiology**

SECONDARY VASODILATION VS EPINEPHRINE REVERSAL IN SKELETAL MUSCLE VASCULAR BEDS. Carlos Rapela*, J.R.R.Bobb and Harold D. Green, Bowman Gray School of Medicine, Winston-Salem, N.C.

The following observations have been recorded with an electromagnetic flowmeter in dogs in response to I.A. injections. Epinephrine and arterenol both caused vasoconstriction followed often by approximately equal degrees of secondary vasodilation; both responses were usually intensified by saturating the beta dilator receptors by I.A. infusion of isoproterenol. Both were diminished by I.A. injection of small doses of adrenergic blocking agents. Larger doses of the latter converted the epinephrine responses to primary vasodilation but only diminished the arterenol constrictor responses; infusion of isoproterenol at this time restored the epinephrine primary vasoconstrictor and secondary vasodilator and intensified the arterenol constrictor and secondary dilator responses. Conclusions: The dilator responses to isoproterenol and to epinephrine during adrenergic blockade (beta receptor activity) appear to be different from the secondary dilation induced by both epinephrine and arterenol. They could be explained by the existence in skeletal muscle of two types of vascular beds (at least partially in parallel); type (M) containing alpha constrictor receptors and capable of secondary vasodilator responses, and type (S) containing beta dilator receptors. Supported by a grant from the A.H.A.

18 **Physiology**

AUTOREGULATION AND PRE- AND POST- GLOMERULAR RESISTANCE CHANGES IN THE ISOLATED PERFUSED KIDNEY. Lerner B. Hinshaw and Nguyen K. Thuong.* Dept. Physiol., Univ. Minn. Med. Sch., Minneapolis, Minnesota.

The objective of the present study was to evaluate the relative roles of pre- and post- glomerular resistance changes in the autoregulation phenomenon. Isolated dog kidneys were perfused at controlled arterial pressures with a pump-lung apparatus. Ureteral pressures were obtained during ureteral occlusion and glucose diuresis, and were assumed to approximate the extravascular pressure in Bowman's capsule. Tissue, ureteral, and intrarenal venous pressures and flows were measured as renal artery pressure was progressively elevated. From ureteral pressure and tissue pressure data and estimates of plasma colloid osmotic pressure (parallel study), calculations of glomerular and peritubular capillary pressures were made. "Overall" (RA-RV/F) and pre- and post- glomerular vascular resistances were calculated throughout the autoregulatory range. Results indicate that progressive increases in post-glomerular resistance are responsible for the total rise in "overall" renal resistance. Pre-glomerular resistance, however, remains constant or falls, as renal artery pressure is increased through the range of autoregulation. These findings are in support of the crucial role of intrarenal extravascular pressure changes in the regulation of renal blood flow.

19 **Physiology**

REACTIVE HYPEREMIA IN THE SEVERELY COOLED FINGER. C.J. Eagan (intr by Homer Wheelon) Defence Research Medical Laboratories, Toronto, Canada.

Evidence has been presented (Eagan, C.J., M.Sc. thesis, U. of West.Ont., 1956) that the initiation of cold-induced vasodilation (CIVD) is due to the interaction of the vascular transmural pressure and the critical closing pressure (CCP) of the arteriovenous anastomoses (AVA). The experiments reported here support the involvement of CCP. On four subjects, in which vascular occlusion (300mm Hg) of one finger was produced for various periods and the corresponding contralateral finger served as control, it was found (with immersion of both fingers in ice-water at 0 min. in all tests) that: a) occlusion from -5 to 0 min. usually, but not invariably, had no effect on CIVD; when an effect occurred it was one of earlier dilation; b) occlusion from -5 to +5 min. had no effect upon the time of dilation but the test finger tended to warm more; c) occlusion from -5 to +1 min. had a marked effect; following release of the occlusion, an increase in finger temperature occurred and there was no phase of intense vasoconstriction, subsequently. This evidence suggests that metabolites, which cause reduction of the CCP of the AVA and resistance vessels, are not flushed out of the finger by the low blood flow existing at +1 min., so that, in spite of the intense cold stimulus, a true reactive hyperemia reaction occurs.

20 **Physiology**

BLOOD FLOW AND OXYGEN UPTAKE RESPONSES TO SHORT PERIODS OF ARTERIAL OCCLUSION. D.I.Abramson, S. Tuck, Jr.* and Y. Bell.*Un. of Ill. Col. of Med., Chicago, Ill.

A study was made of the blood flow "debt" and the oxygen uptake debt incurred in the forearm of 17 normal subjects during 2½, 5 and 10 minutes of arterial occlusion and repaid in the post-occlusion period. Oxygen uptake was calculated on the basis of local blood flow, obtained with the venous occlusion plethysmographic method, and oxygen arteriovenous difference. Except in 1 case, the magnitude of the excess blood flow during reactive hyperemia became progressively greater with prolongation of the period of arterial occlusion. In all instances but 3 the excess oxygen uptake also showed the same type of relationship. For the different periods of occlusion, the observed oxygen uptake was approximately the same as the theoretical oxygen debt incurred in the previous period of arterocclusion(product of the resting oxygen uptake per minute and the number of minutes of anoxia). (Supported by a grant from the U.S.P.H.S.)

21 **Physiology**

RELATION OF PRESSURE TO FLOW IN THE INTACT KIDNEY. R. Hardin, J. Scott and F. Haddy [Intr. by Allen Lein] U. S. Army Med. Res. Lab., Ft. Knox, Ky.

Numerous studies indicate that resistance to blood flow through the kidney increases with increasing arterial pressure over the range 80 to 200 mm.Hg. However, this relationship was not found in a recent study in which the techniques employed for perfusion left the kidney and its associated structures undisturbed [Am. J. Physiol. 197:595, 1959]. Since there are two lumbar arteries which by this technique may have received perfused blood, these have been considered in the experiments herein reported. With both kidneys being perfused, increasing pressure induced an apparent decrease in resistance when the lumbar arteries were patent but caused an increase in resistance in 8 out of 10 experiments when these arteries were clamped. During perfusion of a single kidney, resistance increased in only 4 out of 15 experiments when the lumbar arteries were open but increased in 18 out of 18 experiments when the lumbar arteries were closed. Pressure-flow studies in the lumbar arteries alone revealed a pressure of 195 mm.Hg at a flow of 112 ml./min. Hence autoregulation was demonstrated during aortic perfusion when branches other than the renal arteries were clamped. Whether the autoregulation was related to the dissection necessary to expose these branches requires further study.

22 **Physiology**

CONTROL OF PERIPHERAL VASCULAR BEDS DURING EXERCISE. J.P. Remensnyder*, J.H. Mitchell*, S.J.Sarnoff. Lab. Cardiovascular Physiol., NHI, Bethesda, Md.

Evidence has been obtained which supports the position that, in an area of increased metabolic activity, the regulation of the vascular bed is dominated by local phenomena rather than by central mechanisms. Autoregulation in peripheral vascular beds supplying active areas was examined utilizing measurements from hind limbs of dogs in which exercise was induced by intermittent ramped electrical impulses to the muscles. Continuously recorded parameters were: systemic arterial pressure, heart rate, blood flow to both hind legs, A-V O_2 difference (Guyton), and arterial and venous pO_2 (polarographic) and pH. Measurements were made before and during exercise of both lower extremities and also when one lower extremity was exercised while the other served as a resting control. Sympathetic or sympathomimetic stimuli were applied in three ways:(1) varying carotid sinus pressure;(2)stimulation of the central end of the cut vagus; and (3)intra-arterial injection of norepinephrine. The data obtained indicate that reflexly induced sympathetic stimuli to vasoconstriction are ineffective in the vascular bed of tissue in which the metabolic requirements are increased. The vascular bed in an active area appears to be functionally sympathectomized and relatively unresponsive to sympathomimetic stimulation.

23 **Physiology**

PULMONARY AND CARDIOVASCULAR EFFECTS OF SARIN IN TREATED DOGS. Glenn D. Lubash, Irwin Gribetz, and Rudolph P. Johnson (intr. by William H. Chambers.) Army Chemical Center, Md.

Previous reports have indicated that peripheral vascular collapse is an important cause of death after enormous doses of cholinesterase inhibitors, despite therapy with atropine and artificial respiration. To test this thesis, massive intravenous doses of Sarin (isopropyl methyl phosphonofluoridate), a cholinesterase inhibitor, were administered to 12 dogs in an attempt to induce hypotension. When atropinization and artificial respiration were adequate, blood pressure was maintained even though as many as 500 LD50 of Sarin were used. Intravenous atropine sulfate was also successful in reversing elevated tracheal pressures and heart block induced by Sarin. Metaraminol, norepinephrine, and molar sodium lactate were administered intravenously after treatment with artificial respiration and atropine, and were evaluated with regard to their cardiovascular effects in the poisoned animal. In normotensive poisoned animals given metaraminol, significant complications appeared concomitant with blood pressure rises. In the hypotensive (and hypoxic) animals, metaraminol, norepinephrine, and molar sodium lactate failed to produce satisfactory responses. The value of adequate atropinization and artificial respiration is re-emphasized.

057. CARDIOVASCULAR SYSTEM—Blood Flow—Pulmonary

1 **Physiology**

PHYSIOLOGICAL ALTERATIONS OCCURRING DURING THE DEVELOPMENT OF PULMONARY HYPERTENSION. Lois T. Ellison, David P. Hall, T. Yeh, H. Mobarhan, J. Rossi and Robert G. Ellison (intr. by W. F. Hamilton.) Medical College of Georgia, Depts. of Physiology and Surgery (Thoracic), Augusta, Ga.

Alterations in pulmonary function and hemodynamics were studied in dogs with high flow pulmonary hypertension resulting from surgical shunts in the presence of reduced lung vascular bed. A-aO_2 differences at different levels of oxygenation were determined in 25 controls and at intervals in 30 animals who survived operation an average of 14 months. Repeated cardiac catheterizations were done with determination of flow (Fick) and pressures. Lung biopsies were taken. In 29 dogs systolic pulmonary artery pressure was 40 mm. Hg or more. Abnormal venous admixture occurred in 25 animals and both abnormal venous admixture and diffusion were present in 13 cases. High flow alone without significant pulmonary hypertension often produced abnormal venous admixture, but as a rule pressure exceeded 50 mm. Hg before diffusion difficulty was evident. In 2 cases with pressure over 50 mm. the functional alterations and hypertension persisted after obliteration of the shunts. The pathogenesis of these results will be discussed and correlations with vascular changes presented. (Supported by USPHS grant H-2562, Ga. Heart Assoc. and LIMRF.)

2 **Physiology**

EFFECTS OF CHANGES OF SYSTEMIC AND PULMONARY ARTERIAL AND VENOUS PRESSURES ON BRONCHIAL COLLATERAL FLOW. A. M. Rudolph, P. A. M. Auld* and R. J. Golinko.* Harvard Med. School and Children's Med. Center, Boston, Mass.

A preparation for separate perfusion of the pulmonary and systemic circulations has been devised in anesthetized open-chest dogs. The only remaining communication between the two circulations has been the bronchial collateral circulation. The magnitude of bronchial flow could be measured by recording the gradual and constant change in pulmonary reservoir volume. Increase in systemic arterial pressure by increasing systemic perfusion or by means of phenylephrine increased "forward" bronchial flow. Similarly, an increase of systemic venous pressure by graduated constriction of the systemic drainage tube increased "forward" bronchial flow. Constriction of the pulmonary drainage cannula, with an increase of pulmonary venous pressure produced a decrease in "forward" flow, and further increases resulted in a reversal of flow. An increase of pulmonary arterial pressure alone resulted in a decreased "forward" flow and, when pulmonary arterial pressure was above systemic pressure, in a "reverse" flow. The presence of two bronchial circulations occurring simultaneously has been established by isotope indicator techniques. (Supported by grants from the American Heart Association and the U. S. Public Health Service.)

3 *Physiology*

BLOOD FLOWS IN DEPENDENT VERSUS NONDEPENDENT LUNGS IN HUMANS. Howard B. Keith*, Gunnar Sevelius*, Phillip C. Johnson* and Gilbert S. Campbell. Dept of Surgery and Radioisotope, VA Hosp. and Univ. of Okla. School of Med., Oklahoma City, Okla.

Simultaneous blood flows to similar portions of the right and left lung were recorded in patients in the right lateral decubitus, left lateral decubitus, and sitting positions. Scintillation detectors were positioned against the right and left upper posterior chest wall and the patients were given a rapid intravenous injection of 75 microcuries (0.5 ml. volume) of RISA or radioactive diodrast. Time activity curves were monitored by an ink writing rectillinear recorder. Cardiac outputs were recorded concommitantly by precordial placement of a third scintillation detector in some subjects. The ratios of the blood flow through the dependent and nondependent lung areas were computed. In patients in the right and left lateral decubitus positions the dependent lung area monitored has two to three times the blood flow as has the same area when positioned superiorly. (i.e. in patients without cardiopulmonary disease). The ratio of right to left pulmonary blood flows in areas monitored in sitting patients were 1.2 to 1.0. Resting cardiac outputs were not significantly altered in right versus left lateral decubitus positions. Position changes in pulmonary blood flow are much less in patients with mitral valvar disease and elevated left atrial pressure.

4 *Physiology*

ESTIMATION OF DISTRIBUTION OF BLOOD FLOW IN THE DOG'S LUNG. B. B. Ross and L. E. Husband,* Univ. of Oregon Med. School, Portland, Ore.

The relative rates of blood flow in various lobes of the lung may be estimated by monitoring the change in composition of the gas contained in the lobe following occlusion of the lobar bronchus. By temporarily occluding a bronchus with a balloon tip catheter and aspirating the trapped gas through fast gas analyzers, it is possible to determine the time required to reach a state of constant gas composition and the rate of change in oxygen concentration. The time required to reach constant composition is dependent on the initial gas composition and the rate of blood flow through the occluded segment. The rate of change in oxygen concentration is found to be exponential and dependent on the rate of blood flow. Comparison of these parameters for different lobes permits the evaluation of the distribution of blood flow within the lung.

5 *Physiology*

CHANGES IN COMPLIANCE ASSOCIATED WITH CHANGES IN SHUNT FLOW IN THE LUNG. Leon E. Farhi and Tulio Velasquez (intr. by H. Rahn.) Univ. Buffalo Sch. Med., Buffalo, New York.

Pulmonary shunt flow is increased in dogs after they have been submitted to negative pressure breathing. At the same time total chest compliance is decreased. These two findings are indicative of collapse of alveoli (atelectasis). Anesthetized dogs, paralyzed with succinylcholine and ventilated with oxygen, were subjected for 10 minutes to various degrees of negative intra-alveolar pressure. Five minutes following return to normal pressure the compliance was determined. Simultaneously the venous admixture was calculated from the alveolar and arterial oxygen tensions and the arteriovenous oxygen difference. As the venous admixture increases, the compliance decreases. Changes in the two parameters are not proportional, a decrease of 50% in compliance being associated with about 20% increase in venous admixture.

6 *Physiology*

RADIOACTIVE TRACER DILUTION STUDIES IN THE CANINE HEART-LUNG PREPARATION. D. Tudilevich* and C.W. Sheppard. Dept. of Physiology, University of Tennessee, Memphis, Tenn.

First-pass curves through the lungs and heart were obtained by injection of carrier-free radioactive Na^+, K^+ and I^- into the superior vena cava. Sixty samples of 5-8 ml were collected from the arterial side and the total time of collection was recorded. Arterial and venous pressure recordings indicated the stability of the preparation. Aliquots of hemolyzed samples were used for radioactivity determinations. The fraction of dose after the last sample in the lungs was about 1% for I 131 and 7% for K 42 and in the heart 0.5% for I 131 and 5% for K 42, the blood contribution in both being negligible. For K 42, flow values calculated from the curves of concentration versus cumulated volume gave about 18% larger values than flows measured directly by collection of blood. Central volumes measured from the centroidal values of the curves were not significantly different. It is concluded that under our conditions K^+ moves out of the lesser circulation about 6X faster than I^-. For K 42 the high cardiac outout is a result of incomplete tracer recovery (premature curve truncation). Correction would falsely elevate central volume by including an extravascular component.

Supported by grant # H-3665 from the USPHS.

7 *Physiology*

EFFECTS OF SEROTONIN ON PULMONARY BLOOD VOLUME AND FLOW IN THE DOG. Charles J. McGaff and William R. Milnor (intr. by E. C. Andrus) Johns Hopkins Univ. and Hosp., Baltimore, Md.

Cardiac output, pulmonary blood volume (PBV), pulmonary artery, left atrium, and femoral artery pressures were measured in 8 anesthetized dogs (16 pairs of observations) during control periods and during continuous intravenous infusion of serotonin in doses sufficient to produce a marked increase in pulmonary vascular resistance (0.15 to 1.0 mg/kg/min). Femoral arterial dilution curves were recorded after rapidly consecutive injections of Cardiogreen (R) dye into pulmonary artery and left atrium. The difference in mean transit time (MTT) between the 2 curves was considered pulmonary MTT. PBV was calculated by multiplying cardiac output (ml/sec) by pulmonary MTT (sec). Serotonin increased cardiac output by an average of 8.0%, which would shorten pulmonary MTT by 8.5% if PBV remained constant. Since mean pulmonary MTT actually decreased 34 %, it follows that PBV decreased significantly ($p < 0.01$) during serotonin. Mean control PBV was 112 ml/ 10 kg, mean PBV during serotonin 87 ml/10 kg. Systemic vascular resistance decreased during serotonin. Assuming no change in total blood volume, these results indicate that serotonin-induced pulmonary vasoconstriction is accompanied by a shift of blood from pulmonary to systemic vessels.

8 *Physiology*

A PULMONARY VASCULAR-BRONCHOMOTOR REFLEX. S. Rodbard and M. Harasawa*, Chronic Disease Research Institute, University of Buffalo, Buffalo, N.Y.

Duration of the respiratory cycle of the Burns valve is proportional to insufflation lung volume. In thoracotomized dogs, available volume and cycle duration increase after bronchodilators: aminophylline, TEAC, and adrenalin; decreasing after bronchoconstrictors: histamine, serotonin, acetylcholine, and vagal stimulation. Effective doses for intrabronchial artery injection are only a few percent of that required in systemic administration. Elevation of left atrial pressure(P_{LA}) above 12 mm. Hg by constriction of aorta or pulmonary veins sharply decreased both volume and duration. Threshold P_{LA} was raised by higher insufflation pressures. Saline infusions reduced the blood oncotic pressure and lowered the P_{LA} threshold. Threshold was unaffected by elimination of central nervous system. Effect is not due to volume replacement of tidal volume by fluid since pulmonary vascular engorgement had no effect. Data suggest that receptors in intrapulmonary vessels are activated by capillary transudation, producing an axone reflex increase in bronchomotor tone. In the intact animal this would produce an intrinsic positive pressure expiration, adjust intra-alveolar pressure, transmural capillary pressure, vascular resistance, and ventilatory exchange, protecting against transudation and pulmonary edema.

9 **Physiology**

HEMODYNAMICS OF ALTITUDE HEART FAILURE IN CALVES. H.H. Hecht, H. Kuida, R.L. Lange*, J.L. Thorne*, and A.M. Brown*. Dept. of Med., Univ. of Utah College of Medicine, Salt Lake City, Utah.

Heart failure occurs in cattle above 8000 ft. and is partially reversible at lower elevations (Brisket Disease: Fr. Assoc. Am. Phys. 72:157, 1959) Cardio-respiratory function using right and left heart catheterization in 33 animals (16 controls) studied 96 times revealed: VPRC: Mean 43% SEM±1.4 (Controls: 36% SEM±1.3 $p<0.01$) Sa_{O_2} 78%±2.8 (91±0.7 $p<0.01$) V_{O_2} ml/min/m^2 189±12.9 (356±9.0), $Ca-v_{O_2}$ vol % 6.28±0.32 (4.34±0.13), C.I. l/min/m^2 3.4±0.3 (7.4±0.3) Stroke Vol. ml/beat 62±0.52 (138±3.3), $PaCO_2$ mm Hg 35±0.9 (34±1.3). Pressures (in mm Hg): PA 61±4.3 (21±1.6) RA 32±0.7, left ventricular diastolic 21±2.4. Pulmonary vascular resistance measured in units m^{-4}: 13.3±1.5 (1.9±0.1) Though related to chronic mountain sickness in man the syndrome resembles pulmonary hypertensive heart failure with tricuspid insufficiency. The elevated left ventricular diastolic pressures suggest an additional element of left heart failure. The low oxygen consumption is unexplained.

10 **Physiology**

THE EFFECT OF ACUTE ANOXIA ON PULMONARY HEMODYNAMICS OF THE CALF. H. Kuida, R.L. Lange*, A.M. Brown*, and H.H. Hecht., Dept. of Med., Univ. of Utah College of Med., Salt Lake City, Utah.

The pulmonary vascular response to acute anoxia has been studied in several species with inconsistent results. The occurrence of spontaneous pulmonary hypertension in calves grazing at high altitude provided a basis for specifically examining the response in the bovine species. By means of cardiac catheterization, dye dilution techniques and endotracheal intubation measurements were made of pulmonary and carotid arterial pressures (PAP, CAP) and oxygen saturation (Sa_{O_2}), cardiac index (CI), and calculated pulmonary vascular resistance (PVR) before and after exposure to gas mixtures of 10-16% oxygen in air for 5-20 minutes. 16 anoxic periods were produced in 13 unanesthetized normal calves with a percentage fall in Sa_{O_2} from control levels (87-91%) averaging -33% ± 18. 4 calves developed saturations less than 50% of control. PAP increased in 14 of 15 instances averaging 12 mm Hg or ± 52 of control. CI rose in 8 and fell in 3 animals; the average change was + 13% ± 23. PVR increased in 10 and remained unchanged in 2 studies and with an average percentage increased over control data of + 65% ± 77. This study indicates that in calves acute anoxia is generally associated with an increase in pulmonary vascular tone.

11 **Physiology**

PULMONARY GAS EXCHANGE DURING PARTIAL HEART-LUNG BYPASS IN CLOSED-CHEST DOGS. Max A. Hopf, Pierre-M. Galletti and Naci Bor (intr. by J. Haldi). Dept. of Physiology, Emory University, Atlanta, Ga.

During partial heart-lung bypass, gas exchange occurs simultaneously in the lung and in the pump-oxygenator. At each site the efficiency of gas exchange depends upon the ventilation/blood flow ratio, which can be artificially regulated within wide limits. In this study partial heart-lung bypass (caval veins to femoral artery) was performed in 15 chloralose anesthetized dogs breathing spontaneously. Maintaining a constant body blood volume, extracorporeal flows of 300, 600, 900 and 1200 ml/min were achieved stepwise over a period of 3 hours. Sampling of expired gas, central venous and arterial blood permitted measurements of gas exchange and blood flow in both circuits. With increases in extracorporeal flow, both the pulmonary oxygen uptake and the pulmonary blood flow decreased markedly. Pulmonary ventilation and arterial blood pH remained, whereas arterial CO_2 content decreased by 2-10 vol%. The arterio-venous oxygen difference usually increased by 1-4 vol%. All animals survived indefinitely. It is concluded that partial heart-lung bypass can be performed in spontaneous breathing dogs under maintenance of nearly normal tissue perfusion.

12 **Physiology**

THE EFFECT OF UNILATERAL PULMONARY ARTERY OCCLUSION UPON CALCULATED PULMONARY BLOOD VOLUME. D.S.Dock*, L.B.McGuire*, J.W. Hyland*, F.W.Haynes and L.Dexter. Depts. of Medicine, Peter Bent Brigham Hosp. and Harvard Medical School, Boston, Mass.

The pulmonary blood volume (PBV) was determined (Fed.Proc. 18:84, 1959) in a series of patients with valvular heart disease before and after unilateral pulmonary artery occlusion by balloon catheter. Right heart catheterization was performed with the tip of a triple-lumen balloon catheter placed in left or right main pulmonary artery. The Ross transeptal approach to the left atrium was used. Simultaneous injection of two different indicators was made into pulmonary artery (PA) and left atrium (LA), with sampling from brachial artery. The difference in mean transit times X cardiac output (CO) permitted calculation of PBV. Only slight changes were noted in CO, PA and LA pressures and peripheral arterial O_2 saturation following diversion of the total CO to one lung. Decreases in pulmonary mean transit times of 1.0 to 4.6 sec. were observed, with associated decreases in PBV of 46 to 184 cc. Assuming that pulmonary blood flow and volume were initially evenly divided between both lungs, with doubling of flow through one lung there were associated increases in volume of 27 to 70% in the unoccluded pulmonary vascular bed. It appears that accompanying unilateral PA occlusion, there is an increase in both volume and velocity of flow through the opposite lung.

13 **Physiology**

COMPARISON OF "SLOPE" AND "MEAN TRANSIT TIME" CENTRAL VOLUMES L.B.McGuire*, D.S.Dock*, J.W.Hyland*, F.W.Haynes and L.Dexter. Depts. of Medicine, Peter Bent Brigham Hospital and Harvard Medical School, Boston, Massachusetts.

Analysis of indicator-dilution curves permits calculation of vascular volumes by two methods. The "slope volume", $V_S = F/S$, is derived from blood flow (F) and the slope (S) of the exponential decay portion of the dilution curve. Mathematically, this represents the volume of the largest "chamber" between injection and sampling sites, but the biological identity of such a "chamber" has not been defined in the central circulation. The "mean transit time volume", V_{MTT}, is obtained as the product of the mean transit time (MTT) of indicator and blood flow. Simultaneous injections of different indicators into pulmonary artery (PA) and left atrium (LA), with sampling from brachial artery (BA) has made possible calculation of vascular compartments by the two methods in a series of patients. There was no correlation between V_S from PA injection and V_{MTT} (PA to IA), suggesting that V_S from PA injection is unrelated to pulmonary blood volume (Fed. Proc. 18:84, 1959). Close agreement between the V_S from both PA and LA injection, over a wide range of calculated volumes, indicates that the V_S derived from both curves represents the same biological "chamber", i.e., either LA, left ventricle, or both.

14 **Physiology**

NEW METHOD FOR ESTIMATING PULMONARY CAPILLARY BLOOD FLOW IN MAN. Karlman Wasserman and J. H. Comroe, Jr. Cardiovascular Research Institute, U. of Calif. Med. Sch., San Francisco.

The Lee-DuBois body plethysmograph technic for continuous recording of pulmonary capillary blood flow by N_2O uptake has limited usefulness because the "box" is confining and extremely temperature sensitive. We have eliminated the need of a body plethysmograph by requesting the subject (sitting, standing or exercising) to maintain his thorax at constant volume:- The subject exhales to residual volume, inhales maximally 80% N_2O-20% O_2 or air (control) and exhales to FRC. He then holds his breath with glottis and mouth open to a sensitive Krogh spirometer for continuous record of gas uptake. We monitor chest volume by a sensitive pneumograph-strain gauge and measure N_2O uptake only when chest volume varies no more than 1/4 ml between successive heart beats. As many as 5 measurements have been possible in the subject's first 15 second trial; he improves markedly with practice. A specific R correction is made from control (air) data. In 6 healthy resting men, average cardiac and stroke indices (pulmonary capillary flow) were 3.03 L/min ± 0.42, and 41 ml ± 3.0. Reproducibility is good (4.9 - 5.5 L/min and 72 - 77 ml for a subject studied 4 times at 7-10' intervals). Flow changes have been measured during the cardiac cycle and during exercise. (Supported by USPHS Research Grant H-4029)

XUM

15 **Physiology**

ANALYSIS OF SIMULTANEOUSLY RECORDED ALVEOLAR GAS AND ARTERIAL BLOOD P_{O_2} DURING NITROGEN WASHOUT. T.N.Finley (intr. by B. Libet) Cardiovascular Research Inst., U.Cal.,San Francisco.

During the inhalation of oxygen, end-tidal P_{O_2} and pulmonary end-capillary P_{O_2} can be calculated by using a N_2 meter, infrared CO_2 meter and an O_2 electrode continuously recording arterial P_{O_2}. The latter is corrected for circulation time, instrument lag and shunting. The differences between these tensions and their eventual plateau tensions were plotted logarithmically against number of breaths and analyzed into "fast" and "slow" compartments. The blood curve for the "fast" compartment is distorted by diffusion limitations and unevenness of $\dot{V}_A/\dot{Q}_c$. When the "fast" compartment has completed its washout and the P_{O_2} in the "slow" compartment is high enough to saturate Hb, these distorting effects are eliminated and the two curves become parallel in the "slow" compartment. In the "slow" compartment in 12 normals and 4 asthmatics the curves were parallel and superimposed; in 10 patients with emphysema, 7 with interstitial fibrosis, and 3 with mitral stenosis and pulmonary hypertension the curves were parallel but the blood curve lagged behind the gas curve. This lag of the blood curve is interpreted as being due to over-perfusion of the "slow" compartment caused by an altered vascular bed.

(Supported by USPHS Research Grant H-4029)

16 **Physiology**

CATECHOL AMINES IN THE PULMONARY HYPERTENSION OF ACUTE HYPOXIA. R.M.Goldring, G.M.Turino, G.Cohen, A.G.Jameson and A.P.Fishman (intr. by D.W.Richards), Columbia University, New York, N.Y.

The concentrations of epinephrine (E) and norepinephrine (NE) were measured in the arterial and mixed venous blood of 13 normal subjects, during 3 experimental circumstances: 1) the breathing of 11% O_2 (acute hypoxia), 2) the infusion of NE and 3) hypoxia plus infusion of NE. The measurements included cardiac output (direct Fick), central blood volume (dye dilution) and blood pressures (pulmonary and brachial artery, pulmonary "wedge"). The results indicate: 1) the absence of abnormally high quantities of E or NE during acute hypoxia, 2) the need for a 10 X increase in circulating E or NE to duplicate the pulmonary arterial hypertension of acute hypoxia, and 3) unchanged "wedge" pressures during hypoxia and increased "wedge" pressures during NE infusion. In 2 other subjects, injection of NE into the pulmonary artery during open thoracotomy showed that a rise in left atrial pressure anteceded the rise in pulmonary artery pressure. The pulmonary pressor response to acute hypoxia in man is not related to circulating levels of E and NE.

17 **Physiology**

PULMONARY CIRCULATORY DYNAMICS IN PATIENTS WITH MITRAL VALVULAR DISEASE DURING METHOXAMINE INFUSION Paul N. Yu, C. Alpheus Stanfield, James K. Finlayson and Milton N. Luria (Introduced by J.N. Stannard) Univ. Rochester School of Medicine, Rochester, N.Y.

Changes in the pulmonary artery wedge pressure ("PC") and in the time-concentration components of the indicator dilution curves (IDC) during intravenous Methoxamine infusion were studied in 12 patients with mitral stenosis and 7 with mitral insufficiency. By means of right heart catheterization the indicator was injected into the main pulmonary artery and its concentration recorded from femoral arterial blood. Intravenous infusion of Methoxamine produced: (1) Increased systemic resistance, bradycardia, decreased forward cardiac output; (2) No change in the total and "central" blood volumes; and (3) A delay in appearance and mean transit times. Marked increase in "PC" pressure and its "Y" peak and disproportionate prolongation of the disappearance time of IDC during Methoxamine infusion were found characteristic of mitral insufficiency but not of mitral stenosis. In the patients with mitral insufficiency the characteristic changes were probably due to augmentation of regurgitant stroke volume and alteration of the force of regurgitant jet. It is hoped that by the present method differentiation between predominant mitral stenosis and mitral insufficiency may be made with reasonable certainty so that the left heart catheterization may be obviated.

058. CARDIOVASCULAR SYSTEM—Blood Pressure

1 **Physiology**

NEURAL AND HORMONAL EFFECTS ON SYSTEMIC ARTERIAL PRESSURE. Michael F. Wilson, Neville P. Clarke, Orville A. Smith and Robert F. Rushmer (intr. by N. B. Everett.) Dept. of Physiology & Biophysics, Univ. of Washington School of Med., Seattle, Wash.

Electrical stimulation of discrete diencephalic areas causes reproducible cardiovascular adjustments which may closely simulate responses to spontaneous exercise. Such stimulation produced tachycardia, elevated systolic and diastolic systemic arterial pressures, increased pulse pressure and increased discharge over the splanchnic nerve. When diencephalic stimulation began, splanchnic nerve discharge increased immediately. When central stimulation ceased, the splanchnic activity promptly decreased below the control level and then gradually ascended to it. Injection of l-epinephrine was followed by bradycardia and diminished discharge of the splanchnic nerves. After a large injection of l-epinephrine (30 µg./kg.) systemic arterial pressure remained very high for several minutes and then fell progressively to levels below the control. The splanchnic nerve discharge returned to the control level. Diencephalic stimulation 7 minutes after this injection produced a massive discharge over the splanchnic nerve, but the systemic arterial pressure was not significantly affected. (Supported by Cardiovascular Training Grant 5Ш7 from the National Heart Institute.)

2 **Physiology**

ARE THERE PRESSORECEPTORS IN THE MESENTERIC ARTERIAL BED? G. O. Boyer and A. M. Scher (intr. by H. S. Bennett.) Dept. of Physiology & Biophysics, Univ. of Washington School of Med., Seattle, Wash.

A recent paper by Sarnoff and Yamada (Circulation Res., I: 325, 1959) has re-opened the question of the existence of receptors in the mesenteric-arterial bed. In our investigation, wide variations in pressure were imposed on the mesenteric-arterial system and the systemic response to these was monitored in animals under very light chloralose. In 12 cats and 2 dogs, which had significant responses to alterations in carotid sinus pressure, wide variations in the pressure in the superior mesenteric system produced no significant reflex change in blood pressure. In contrast, the systemic pressure response to occlusion of the mesenteric arteries was similar to that seen during carotid artery occlusion. However, spinal cord section at C_1 or intravenous perfusion of large amounts of sodium pentothal, both of which obliterated carotid pressoreflexes, either did not alter or increased the response to mesenteric arterial occlusion. These results indicate that pressure responses to alterations in mesenteric blood pressure result from mechanical diversion of blood from these arteries into the remainder of the arterial system.

3 **Physiology**

SERVOANALYSIS OF THE CAROTID SINUS REFLEX. A. M. Scher. Dept. of Physiology & Biophysics, Univ. of Washington School of Med., Seattle, Washington.

A pressure-controlled pump delivered sine waves of pressure to the isolated carotid sinus. The input variables are the mean pressure level and the frequency and amplitude of the sine wave. The output variables are the mean level, amplitude and phase difference of the systemic pressure response. Certain consistent results have been noted. In dogs anesthetized with barbiturates or chloralose the open-loop gain of the carotid sinus reflex is less than 2. In cats the gain has been as high as 10; values of 5 have been common. Only when the gain is this high is the reflex a good regulator. In dogs the fall in systemic pressure is in phase with the rise in carotid sinus pressure at low frequencies (.01 c.p.s.); the phase lag is about 90° at frequencies of .04 c.p.s. or more. In cats the phase lag at higher frequencies is as much as 180°. The effectiveness of the reflex is diminished by artificial ventilation, but it is not clear whether lung inflation or hypocapnia is responsible. The infrequent changes in heart rate are out of phase with blood pressure changes. Heart rate changes show time-derivative effects of input pressure which are not prominent in blood pressure changes. In this preparation changes in heart rate apparently are not important regulators of systemic pressure. The reflex response is nonlinear.

4 **Physiology**

BAROCEPTOR REFLEX MECHANISMS IN SWINE. N. H. Booth, H. E. Bredeck and R. A. Herin (intr. by C. A. Maaske.) Dept. of Physiol., Colo. State Univ., Ft. Collins, Colo.

Unilateral and bilateral occlusion and desocclusion of the common carotid artery was performed on intact and bilaterally vagotomized pigs to determine the effect on arterial pressure. A similar procedure was employed following unilateral and bilateral denervation of the sinus region. The effects of carotid occlusion and desocclusion on cardiac rate were recorded prior to vagotomy and the cardiac response to vagotomy was also determined. The results of this study definitely indicate that swine exhibit cardiovascular reflexes initiated by baroceptors located in the carotid sinus area. Evidence was also obtained which supports the existence of cephalic baroceptors. (Supported by a grant from the United Fund of Ft. Collins, Colo. and the Colo. Heart Assoc.)

5 **Physiology**

DIRECT RECORDING OF KOROTKOFF SOUNDS IN MAN. David H. Lewis, Sami Abou Khalil*, and John D. Wallace*. Div. Cardiol., Phila. Gener. Hosp., Phila., and U. S. Naval Air Development Center, Johnsville, Pa.

Using the phonocatheter developed in this lab., direct recording of Korotkoff sounds from brachial artery in man with vascular pressure, cuff pressure, and external sounds has been carried out. Lowering cuff pressure from above systolic shows onset of intravascular sounds with the first pulse of blood through the cuff. External sounds appear at this or next lower beat. Sounds occur at the time of pressure rise in the collapsed vessel. As cuff pressure is lowered external sounds usually disappear before the diastolic pressure is reached. Internal sounds disappear at the diastolic level. Recordings of internal sounds from varying positions within the artery show sounds directly beneath or distal to the cuff but not proximal to it. It is suggested that the Korotkoff sounds are related to the sudden opening of the vessel and that the discrepancy between direct and indirect blood pressure measurement may be related to transmission rather than production of Korotkoff sounds. In aortic insufficiency internal sounds are heard unrelated to cuff pressure.

6 **Physiology**

A COMPENSATING AMPLIFIER FOR THE CORRECTION OF DISTORTION IN PRESSURE MEASUREMENTS. Frank W. Noble* and Octo Barnett* (intr. by Donald L. Fry) National Institutes of Health, Bethesda, Md.

Using the typical liquid-filled catheter-manometer system, there is significant amplitude and phase distortion of the harmonic content in the recorded cardiovascular pressure pulse wave form. The hydraulic behavior of such a catheter-manometer system is similar to the electrical behavior of a simple network consisting of a resistor, inductor and capacitor connected in series.

Based on the foregoing, a compensating amplifier consisting of a variable resistor in parallel with a variable series resistance-inductance-capacitance circuit was designed. These variables are set according to values calculated from the measurement of three points on the frequency response curve of the particular catheter-manometer system.

The use of this compensating amplifier results in both a constant amplitude and a linear phase response to frequencies up to the resonant frequency of the catheter-manometer system. Several examples will be presented to illustrate the importance of its use in both left and right heart catheterizations, as well as in measuring aortic blood pressure, and the instantaneous aortic pressure gradient.

7 **Pharmacology**

RAT AORTA AND MUSCLE ELECTROLYTES FOLLOWING CHLOROTHIAZIDE ADMINISTRATION. W.R. Beavers. Univ. of Texas Southwestern Medical School, Dallas, Texas.

Since this laboratory has previously made observations that chlorothiazide decreases vascular reactivity in the dog and lowers plasma potassium in the nephrectomized dog, it was considered pertinent to determine whether chlorothiazide alters electrolyte concentrations in rat aorta and skeletal muscle. Two groups of ten 200 gm. Holtzman rats were used, one receiving chlorothiazide 30 mg/kg by stomach tube twice daily for four and one-half days, the controls receiving an equal amount of distilled water for a like period. The animals were sacrificed and the plasma, aorta, and abdominal skeletal muscle were analyzed for Na, K, and Cl. Chlorothiazide treated animals had lowered plasma potassium values (4.2 vs 5.1 mEq/l). The aorta potassium was significantly lower than controls (130 vs 147 mEq/kg fat free dry wt.) as was the aorta sodium (75.1 vs 84.4 mEq/kg fat free dry wt.). Other electrolyte concentrations were not significantly different from controls. These data suggest that chlorothiazide may exert a greater effect on aorta sodium and potassium concentrations than on those of skeletal muscle. (Supported by a grant from Natl. Heart Inst., PHS.)

8 **Pharmacology**

A NEW METHOD FOR THE INDIRECT RECORDING OF BLOOD PRESSURE IN RATS. B. Korol, W. McShane, L. Mulieri and H. Weaver, (intr. by S. Y. P'an) Research Laboratories, Chas. Pfizer & Co., Inc. Groton, Connecticut

Utilizing the principle of oximetry this technic yields reliable determinations over wide ranges of blood pressures. After the rat is placed in a leather holder with the hind limbs free, a cuff is wrapped around an ankle and attached to an 'Infraton' pressure-marking device. The 'Earpiece Oximeter' is placed over the fleshy portion of the paw. Recordings of relative HbO_2 concentrations are made through an Ensco Amplifier. After one minute occlusion the oximeter light is activated and the pressure in the cuff reduced at a rate of 2 mm. Hg per second. Mean arterial pressure is indicated by the directional reversal of the HbO_2 concentration curve when arterial pressure supersedes the applied pressure in the cuff. Correlation studies in anesthetized rats using direct (carotid) and indirect (oximeter) blood pressure determinations show linear parallelism although the indirect measurements appear to be about 10 mm. Hg below the direct determinations. Preliminary correlative studies of simultaneous direct (aortic intubation, Still et. al., J. Appl. Physiol., 8:575, 1956) and indirect (oximeter) arterial pressures in unanesthetized rats indicate similar parallel relationships.

9 **Pathology**

A NEW METHOD FOR IN UTERO FETAL OPERATIONS: CHRONIC BLOOD VESSEL CANNULATION. B.T. Jackson* and R.H. Egdahl. Surg. Res. Lab., Med. Coll. Va., Richmond, Va.

Most previous studies in fetal physiology have been associated with considerable mechanical alterations in fetal-maternal relationships. The technique described herein allows for extensive and detailed fetal surgery with a minimum of these disturbances. The uterus of the pregnant dog is exposed and the fetus is ballotted against its wall. The skin of the fetus and the uterine wall are stitched together in an elliptical pattern and an incision made within the ellipse through the uterus and into the fetus, creating in effect a marsupialization of the fetus. The operative procedure is then carried out in a conventional manner, with exposure and cannulation of the desired blood vessel or vessels, eg., the carotidartery, portal vein, or inferior vena cava. Cannulas thus implanted may be maintained for chronic pressure recordings, blood samplings, or injections by leading them to the outside of the mother following closure and separation of the uterus and fetus. Fetal adrenal venous blood samples have been obtained by distal cannulation of the cava, with occlusion proximal to the adrenal with a choker.

XUM

059. CARDIOVASCULAR SYSTEM–Blood Pressure–Hypertension

1 **Physiology**

CARDIOVASCULAR RESPONSES TO STARVATION AND REALIMENTATION. G. S. Smith, M. S. Mameesh, and B. Connor Johnson (intr. by R. E. Johnson). Dept. of Animal Science, Univ. of Illinois, Urbana.

Four pigs were employed in a long-term study of the cardiovascular responses to realimentation following starvation. Drastic changes, in terms of increased heart rate, blood pressure and electrocardiographic patterns, were elicited during realimentation with a balanced diet high in carbohydrate, with pure glucose, and with glucose plus sodium chloride. Neither pure starch, pure protein, nor pure fat regimens elicited comparable responses. Refeeding with glucose alone resulted in a marked increase in heart rate above the pre-starvation level after a short (8-day) but not after a long (30-day) starvation period. Addition of NaCl (2%) to the pure glucose refeeding regimen suppressed the increase in heart rate but raised systolic pressure over the pure glucose refeeding level. Glucose plus NaCl (4%), after an 8-day starvation, raised systolic pressure to hypertensive levels (above 220 mm Hg) and pulse pressure above 60 mm Hg, without noticeable increase in heart rate. A natural corn-soybean diet was used to "standardize" the animals between starvation-refeeding episodes. Systolic pressures recorded during successive standardization periods were found to increase as the experiment progressed.

2 **Physiology**

STUDIES ON EXPERIMENTAL RENAL HYPERTENSION IN RABBITS. C.Giordano*, A.H.Samiy*, J.Bloom*, F.W.Haynes*, and J.P.Merrill. Peter Bent Brigham Hospital; Harvard Med.Sch. Boston, Mass.

Hypertension was produced by clamping of one renal artery one week after contralateral nephrectomy. One hundred and twenty animals were employed 10 per cent of which developed persistent systolic hypertension in absence of azotemia. Group I was sacrificed one week after and group II 8 weeks after the onset of hypertension. Kidney monoamine oxidase and dopa decarboxylase activities were manometrically assayed. Plasma renin level was determined in all animals. Rabbit antirenin was injected intraperitoneally in some animals. Plasma renin levels increased markedly during the early phase of hypertension and then gradually fell to normal levels. The adminstration of antirenin induced a hypotensive effect only during the first week of hypertension. Kidney monoamine oxidase and dopa decarboxylase activities were within normal limits in the first group of animals. However, during prolonged hypertension there was an increase in the activity of dopa decarboxylase and a diminution in that of monoamine oxidase. These data suggest that the early phase of renal hypertension in the rabbit is probably induced by increased production of renin and that the late phase of renal hypertension is associated with a change in the activity ratio of these enzymes.

3 **Physiology**

STUDIES OF RENIN AND JUXTAGLOMERULAR CELLS, COMPONENTS OF A REGULATING SYSTEM IN THE KIDNEY. J. Janecek*, L. Tobian, M. Otis *, J. Hunieke*. Dept. of Medicine, Univ. of Minnesota.

The granularity of rat juxtaglomerular cells (the juxtaglomerular index) is normally 30. The extractable renin in such a normal kidney will cause a 37 mm. rise in blood pressure in a bioassay rat. If a clip is made to narrow one renal artery so that hypertension results, the juxtaglomerular index of the opposite kidney falls to 1 and there is not enough extractable renin in that kidney to produce any hypertensive response at all. If the clipped kidney is excised and the hypertension becomes "cured", the juxtaglomerular index in the opposite kidney rises to 27 and the extractable renin in that kidney also increases greatly so that it produces a 28 mm. rise in blood pressure in a bioassay rat. In another experiment, one group of normal rats was fed chow containing 7.4 % NaCl. The other group was fed ordinary chow only. Both groups drank tap water ad libitum. The kidneys of rats on ordinary chow had an average JG index of 29 and an average renin response of 45 mm. Hg. The kidneys of rats on chow with 7.4 % NaCl had an average JG index of 15 and an average renin response of 27 mm. Hg ($p < .02$). In all these rats there was a strong correlation between the renin content and the juxtaglomerular index in the individual rat kidneys, suggesting that renin is the secretory substance of the JG cells.

4 **Physiology**

EFFECT OF NEPHRECTOMY ON THE PRESSOR RESPONSES TO RENIN, ANGIOTENSIN AND NOREPINEPHRINE IN THE RAT, John Schroeder*, Pedro Blaquier*, Sibley W. Hoobler and David F. Bohr, Depts. of Int. Med. and Physiology, U. of Mich., Ann Arbor, Mich.

Pressor responses to norepinephrine, synthetic angiotensin and hog renin were elicited by intravenous injection of the several materials in rats before and after nephrectomy. Twenty-four hours without renal tissue did not significantly alter the response to angiotensin or norepinephrine, but did substantially augment the pressor response to renin. Response to renin was not significantly changed ± 20 minutes after nephrectomy; it was slightly potentiated at 4 hours, maximally potentiated at 7 hours, and this maximum potentiation was maintained at 24 hours post-nephrectomy. When 24-hour post-nephrectomized rats were cross-circulated with normal rats, the injection of renin caused similar responses in the two rats. This suggests that the potentiation of the response to renin may have been mediated through some humoral mechanism. It was further found that the pressor response of rats to renin was increased by injection of plasma taken from rats 24 hours after nephrectomy as compared with injection of plasma from normal rats. Our conclusion is that the potentiation of the renin activity by nephrectomy is the result of the accumulation of a potentiating substance in the blood following bilateral nephrectomy. (Supported by grant H2578C3 from the Natl. Heart Inst. P.H.S.)

5 **Physiology**

USE OF THE PITHED RAT IN EVALUATION OF THE MECHANISMS OF EXPERIMENTAL RENAL HYPERTENSION. Alberto C. Taquini, Jr.*, David F. Bohr, Pedro Blaquier* and S. W. Hoobler. Depts. of Physiol. and Int. Med., Univ. of Michigan, Ann Arbor.

Pithing was orbital and extended the length of the cord. Average mean pressures of 3 groups of anesthetised, artificially respired rats were recorded:

Group	No. of animals	Pre-pith	Post-pith
A. Normal	11	143.9	31
B. Hypertensive	5	181.2	34
C. Angiotensin-Infused	5	131 (pre-inf)	52 (infused)
		146.4 (infused)	30 (post-inf)

There was no significant difference between the post-pith pressures of normotensive and renal hypertensive rats. Following pithing the heart rate fell and central venous pressure rose suggesting that the primary determinant of post-pith pressure might be a decreased C O resulting from a hypodynamic heart. However, the failure of the pressure to reach a similar floor when the rat was pithed during an angiotensin-induced pressure elevation argues that the heart was capable of maintaining an increased pressure in the presence of an increased TPR. This evidence strongly suggests that renal hypertension is not maintained by circulating angiotensin alone. (Supported by grant H2578C3 from the Nat'l. Heart Inst. PHS)

6 **Physiology**

INSTANTANEOUS DIMENSION-PULSE AND MEAN DIMENSIONAL CHANGES IN THE KIDNEY OF AWAKE DOGS. G.V. Brownlee and Edward W. Hawthorne (intr. by Joseph L. Johnson) Howard University School of Medicine, Washington, D. C.

Our previous studies suggest that the initial pathogenic mechanism in experimental renal hypertension is a decrease in the instantaneous dimension-pulse of the kidney. A means has been devised for recording the instantaneous dimension-pulse and mean dimensional changes in the kidney of awake, intact dogs. Sufficient amplitude is achieved so that instantaneous changes in the dimension-pulse can be perceived with facility. This method utilizes a variable resistance gage (Whitney). It forms one leg of an AC bridge circuit activated by the carrier wave of a Sanborn strain gage amplifier. The gage is applied with slight tension to the long arc of the kidney cortex. Recordings from the awake animals show an increase in length of the gage as the volume of the kidney increases with each cardiac systole. Preliminary studies show that the intravenous injection of epinephrine in total doses as low as 0.3 micrograms per kilogram of body weight causes a significant decrease in the mean dimension and dimension-pulse of the kidney of awake dogs.

060. CARDIOVASCULAR SYSTEM—Blood Pressure—Shock

Pharmacology

THE INFLUENCE OF DIURETICS ON VASCULAR REACTIVITY. A. A. Rubin, S. C. Beauregard and B. G. Freeman (intr. by F. E. Roth.) Schering Corp., Bloomfield, N. J.

Although the antihypertensive effects of certain diuretic agents have been clearly demonstrated in man and in laboratory animals, the mechanisms underlying these effects are still under investigation. One concept that has received considerable support implicates transmembrane cationic gradients. As a preliminary approach to this problem, it was considered of interest to determine whether diuretic agents directly affect the responsiveness of vascular smooth muscle. The results so far obtained show that chlorothiazide, 6-chloro-3-dichloromethyl-3,4-dihydro-7-sulfamyl-1,2,4-benzothiadiazine-1,1-dioxide (DCH), aminophylline, aminoisometradine, acetazolamide and meralluride in concentrations ranging from 10^{-3} to 10^{-5} (w/v) depress the norepinephrine-induced contractions of *in vitro* rat aortic strips. In preliminary experiments, DCH depressed the aortic contractions induced by serotonin to the same extent as those caused by norepinephrine. Confirmatory data for the DCH inhibition have also been observed in the perfused rabbit ear preparation.

Physiology

LEVELS OF PLASMA BOUND-IRON IN EXPERIMENTAL SHOCK IN THE RABBIT AND DOG. A. Janoff and L.R. Shapiro (introd. by S. Baez). N.Y.U. College of Medicine, New York, N.Y.

Plasma iron was measured in rabbits subjected to lethal doses of endotoxin or infusion of lethal amounts of catechol amines, and in dogs in superior mesenteric artery (SMA)-occlusion shock and compared with plasma iron levels in rabbits and dogs shocked by hemorrhagic hypotension. In both species hemorrhage caused marked elevations in plasma iron, but iron was depressed in rabbits treated with endotoxin or lethal infusions of catechol amines and in dogs shocked by SMA-occlusion. In rabbits this depression was temporarily reversed by reticulo-endothelial (RE) blockade. Plasma uric acid was not depressed in endotoxin treated rabbits. Hemodilution, though present, was not sufficient to account for the observed decreases in iron. It is concluded that enhanced uptake of circulating iron by RE elements is a regular feature of normovolemic shock, while, in hypovolemic shock, tissue hypoxia in liver and spleen reverses this effect. (Supported by grant # DA 49-007-993, Department of the Army.)

Pharmacology

THE EFFECTS OF A NEW BENZOTHIADIAZINE DIURETIC AND RESERPINE ON AMINES OF BRAIN AND HEART. I. I. A. Tabachnick and H. Beil*. Schering Corp., Bloomfield, N. J.

Many clinical reports have demonstrated that chlorothiazide enhances the effects of drugs which depress the autonomic nervous system, i.e., reserpine, hexamethonium, and mecamylamine. We have studied the effects of reserpine, a new diuretic, 6-chloro-3-dichloromethyl-3,4-dihydro-7-sulfamyl-1,2,4-benzothiadiazine-1,1-dioxide (DCH), and combinations of reserpine and DCH on the amine levels of brain and heart as a measure of potentiation. Subcutaneous doses of DCH, 1 hour, 3 and 6 days before a subcutaneous dose of reserpine (5 mg/kg, 1 hour before autopsy) showed greater depression of norepinephrine (NE) of heart and brain and 5-hydroxytryptamine (5-HT) of brain than obtained with reserpine alone. The most pronounced effects were obtained in the heart. DCH alone had variable effects on NE and 5-HT which were not significant. Comparisons with other diuretics will be developed.

Physiology

VASOACTIVE AGENTS IN PORTAL BLOOD DURING HEMORRHAGIC SHOCK. Carl F. Rothe* and Ewald E. Selkurt. Dept. Physiol., Indiana Univ. School of Med., Indianapolis, Indiana.

Vasoactive agents released from the intestine during hemorrhagic shock in dogs were studied by perfusing either an isolated terminal loop of ileum or an isolated gracilis muscle with oxygenated portal blood at 100 mm Hg. pressure. At 10 to 20 minute intervals, femoral arterial blood at 100 mm Hg. pressure was used as the perfusate to evaluate changes in the preparation and to look for vasoactive agents in the systemic blood supply. At the end of the 40 mm Hg. hypotensive period, vasodilators were consistently found in the portal blood. After transfusion of the shed blood, vasoconstrictors usually seen in the arterial blood during the hypovolemic phase were replaced or masked by vasodilators. However, during the remainder of the normotensive period, vascular tone slowly returned to near normal. Terminally, vasodilators were often seen in both arterial and portal blood. Results with muscle were similar to those using an intestinal loop. Although the degree and pattern of vasodilatation does not appear to be adequate to explain the eventual circulatory collapse, it is suggested that the vasodilatation during the last phase of hypovolemic hypotension may be indicative of the release of agents which act on the cardiovascular system to contribute to irreversibility. (Supported by PHS grant H4056C1.)

Nutrition

HYPERTENSION, DIET AND LIPEMIA IN THE RAT. J. B. Allison, P. Brand*, R. W. Wannemacher, Jr.,* and L. P. Parmer.* Bureau of Biological Research, Rutgers, The State University, New Brunswick, N. J.

Weanling rats were divided into two groups, one receiving 18% casein and the other 40% wheat gluten in a semi-synthetic diet. Lard represented 45% of the caloric intake. After feeding these diets for 28 days, rats from both groups were made hypertensive by unilateral nephrectomy and the administration of deoxycorticosterone acetate (DCA). Appropriate controls were established. The hypertensive rats developed hypertrophy of the heart, liver, and kidney, with a reduced serum albumin : globulin ratio. Serum cholesterol, phospholipid, and lipid migrating electrophoretically with alpha and beta globulins increased markedly above control values. Similar results were obtained in hypertensive adult rats fed these same diets. The increase in serum lipids was most marked in young or adult rats fed the wheat gluten diet. There was even a significant increase in rats with a normal blood pressure but with the unilateral nephrectomy when they were fed wheat gluten. Hypertension could not be produced in rats fed a protein-free diet although increase in serum lipids was associated with depletion in body proteins.

Physiology

TOLERANCE OF SYMPATHECTOMIZED DOGS TO HEMORRHAGE. Shu Chien and Bernard Hitzig (intr. by M.I. Gregersen). Dept. of Physiology, Columbia University, New York.

Total thoracolumbar sympathectomy and splenectomy were performed on dogs (7-12kg) at least 6 wks. before the hemorrhage experiments. Cell volume (Cr^{51}) and plasma volume (T-1824) were measured before and after a known amount of hemorrhage at 100 ml/min. without general anesthesia. Fifty percent survival (or LD_{50}) was found at a residual blood volume of 72% (or 57 ml/kg) in these dogs. Similar experiments performed on splenectomized dogs with the sympathetics intact showed that LD_{50} occurred at a residual blood volume of 61% (or 49 ml/kg)(Rawson, Chien, Peng & Dellenback, Am. J. Physiol.196:179, 1959). The residual blood volume included volume replaced by hemodilution which amounted to an average of only 6% of the initial blood volume in sympathectomized-splenectomized dogs, as compared to 11% in splenectomized dogs. Hence, the amount of blood withdrawal which gave LD_{50} was about 34 [= 100 - (72 - 6)] percent of blood volume in the former group, as compared to 50 [= 100 - (61 - 11)] percent in the latter group. It is concluded that the presence of sympathetic system increases the tolerance to hemorrhage by 16% of the initial blood volume.

XUM

4

ADRENAL CORTICOIDS IN ISCHEMIC SHOCK INDUCED BY TEMPORARY OCCLUSION OF THE THORACIC AORTA IN DOGS. S.S. Levin,* D.Y. Cooper, H.M. Vars, and W.M. Parkins. Harrison Dept. Surg. Res., U. of Pa., Sch. of Med., Phila., Pa.

During occlusion the blood pressure, adrenal blood flow and steroid output (by chromatography and Silber-Porter methods) were reduced to 12%, 2% and 1% of initial, respectively. Following release of one hour of occlusion these respective values immediately increased to 68%, 36% and 36%; but within 3 hours steroid output had declined to 16% as circulation failed. Peripheral blood levels were only moderately decreased in spite of the marked secretory deficiency. No significant quantitative alteration in the pattern of secretion of cortisol, corticosterone and 11-beta-hydroxyandrostanedione was observed. Hydrocortisone (F), methylprednisolone (MF), and 2-methyl 9-fluoro-hydrocortisone (2M9FF) increased survival rate from 20% in the controls to 40%, 60% and 80% respectively of dogs occluded for 60 minutes. Plasma volume and blood pressure were better maintained in the treated than in controls. With more severe ischemia (90 min occlusion) MF and 2M9FF increased survival time but not the number of 72-hour survivors. (Supported by Army Contract No. DA-49-007-MD-511).

5 **Physiology**

A CONTINUOUS RECORDING OXYGEN DEBT ANALYZER. Jack W. Crowell. (intro. by L. H. Schneyer) Dept. of Physiology and Biophysics, Univ. Med. Center, Jackson, Miss.

A continuously balancing flowmeter system is used to regulate the amount of air flowing through a lung tube, and from this tube, near the flowmeter, a side arm is connected to the trachea of a dog. A Beckman Oxygen Analyzer determines the percentage of oxygen remaining in the expired air. With the flowmeter set at a given amount of air flow, and the oxygen analyzer set at 1 volt change for each 1% change in oxygen, the amount of oxygen being used by the dog is continuously determined. This signal is transmitted to a servoamplifier through a back EMF circuit so that any portion of the signal may be balanced out. With the dog breathing normally, the back EMF circuit is set so that a zero signal is fed to the servoamplifier. Any changes from normal cause the servoamplifier to deflect. The servoamplifier is used to set the deflection of a ball and disc integrator system. Both the servoamplifier deflection and the integral are continuously recorded. This instrument has been used in the study of oxygen debt in hemorrhagic shock.

6 **Physiology**

ACTIVATION OF RAT SERUM PROTEOLYTIC ACTIVITY FOLLOWING NOBLE-COLLIP TRAUMA. Letitia Beard* and John K. Hampton, Jr.

We have found that Noble-Collip Drum trauma produces statistically significant increases in serum proteolytic activity of traumatized normal and trauma-resistant rats. Trauma resistance was produced by progressive sublethal exposures to trauma and proteolytic activity was measured by the ability of test samples of serum to release TCA soluble fractions from I^{131} tagged casein. The increased proteolytic activity was directly correlated with the dose of trauma administered. The trauma-resistant rats exhibited an increment in serum proteolytic activity of smaller magnitude than normal rats following trauma. (Supported by a grant from the USPH).

7 **Physiology**

COMPARISON OF ALTERATION IN INTESTINAL WEIGHT DURING ENDOTOXIN SHOCK AND PARTIAL PORTAL VENOUS OCCLUSION. M. W. Meyer* (intr. by M. B. Visscher) Dept. of Physiol., Univ. of Minn. Med. Sch., Minneapolis, Minnesota.

Intravenous administration of E. coli endotoxin to the dog produces a drop in systemic pressure, an increase in portal venous pressure, and an increase in intestinal weight (Proc. Soc. Expl. Bio. and Med., 92:602, 1956). Experiments were performed on dogs anesthetized with nembutal. Time averages of intestinal weight and portal pressure changes during the portal hypertensive period of endotoxin shock (10 minutes) are compared to those observed following partial occlusion of the portal vein for 10 minutes. The average increase in portal pressure for the first 10 minutes after endotoxin was 5.4 mm Hg and the average increase in intestinal weight was 5.1%. Portal pressure by partial occlusion of the portal vein was elevated to an average of 14.3 mm Hg and intestinal weight increased 3.9%. This represents a 0.95% increase in weight per mm Hg rise in portal venous pressure in the endotoxin experiments and only 0.27%/mm Hg with partial occlusion. Mechanisms other than elevation of portal venous pressure must play a more important role in the intestinal response observed during the portal hypertensive period of endotoxin shock.

8 **Physiology**

HEMODYNAMICS OF THE CANINE SMALL BOWEL DURING SHOCK. J. K. Longerbeam and R. C. Lillehei (intr. by C. Fahr) Dept. of Surg., Univ. of Minnesota Medical School.

Profound changes were found to occur in the circulation in canine bowel during hemorrhagic and endotoxin shock. Hemorrhagic shock results in an increasing resistance and a decreasing blood flow. The intestinal blood flow is usually < 20% of control flow when B. P. reaches 40 mm. Hg. After short periods of shock, blood replacement rapidly restores both B. P. and intestinal blood flow to normal. Following severe shock (> 30 min.) blood replacement may not restore the B. P. to normal, but blood flow may increase to 150% of normal, probably representing a reactive hyperemia. Endotoxin shock also results in increasing resistance and decreasing intestinal blood flow. Support of the B. P. during endotoxin shock by repeated transfusions results in partial recovery of blood flow to be followed later by a progressive decline in flow until death. Similar and characteristic changes are known to occur in the small bowel as a result of severe shock of various causes. The above study suggests that in endotoxin and hemorrhagic shock the changes in blood flow may be a common denominator which results in the small bowel pathology and contributes to the irreversible state. (Supported by USPHS and Giannini Foundation Grants.

9 **Pharmacology**

EFFECTS OF TRIS (HYDROXYMETHYL) AMINOMETHANE (THAM) ON ACID-BASE DERANGEMENTS AND VENTRICULAR CONTRACTILE FORCE CHANGES ELICITED BY PERIODS OF REDUCED CIRCULATING BLOOD VOLUME. E.L. Aldinger*, T. D. Darby and W. B. Thrower*. Depts. of Pharmacology and Surgery, Medical College of South Carolina, Charleston.

The circulating blood volume was reduced for periods of 10 to 40 minutes in 24 anesthetised open chest dogs. Arterial blood pressure (BP), ventricular contractile force (VCF), arterial pH and plasma CO_2 combining power were determined. Low total body perfusion rates were produced by 1) limiting venous return to that of the azygos vein or 2) regulating extra corporeal circulation with a Mark-Kay-Cross type oxygenator and pump system. Following the interval of hypotension a period of marked metabolic acidosis and a 3 to 7 minute period of increased VCF and BP occurred. Correction or prevention of the metabolic acidosis by the administration of THAM prolonged this "rebound" increment in VCF and BP. The longer interval of stimulation was similar to previously reported intervals of elevated catechol amine levels. In animals with normal acid-base balance THAM produced vasodilation and a slight increase in VCF. Central nervous system damage occurred in some survival experiments. (Supported by grants from National Heart Institute, S. C. Heart Assoc. and Am. Heart Assoc.)

11 *Pharmacology*

EFFECTS OF TRIS (HYDROXYMETHYL) AMINOMETHANE (THAM) ON VENTRICULAR CONTRACTILE FORCE CHANGES ACCOMPANYING LACTIC ACID INFUSION OR ELEVATION OF VENTILATION CO_2. T. D. Darby, E. E. Aldinger*, W. B. Thrower* and S. H. Westbrook*. Depts. of Pharmacology and Surgery, Medical College of South Carolina, Charleston.

In 25 anesthetised open chest dogs, acid-base derangement was produced by the administration of intravenous lactic acid or by increasing the concentration of CO_2 in the ventilation gas. Arterial blood pressure (BP), ventricular contractile force (VCF), arterial pH and plasma CO_2 combining powers were measured. Acidosis was accompanied by a decrease in VCF and a reduction in the inotropic response to injections of levarterenol. An intravenous infusion of THAM was effective in raising arterial pH, increasing plasma CO_2 combining power, increasing VCF and restored or increased the response to injections of levarterenol. In other preliminary studies THAM has proved effective in combating or reversing ventricular fibrillation occurring with periods of asphyxia. THAM has been used to correct metabolic acidosis observed in 5 patients receiving levarterenol. Ventricular fibrillation during thoracotomy operations has been successfully treated with THAM. (Supported by grants from National Heart Institute, S. C. Heart Assoc. and Am. Heart Assoc.)

12 *Pharmacology*

EFFECT OF CHLORAMPHENICOL, GAMMA GLOBULIN, AND CONVALESCENT SERUM ON THE DELAYED DEATHS OF MICE AFTER EXTENSIVE BURN INJURY. R. C. Millican and J. D. Rust*. Natl. Insts. of Health, Bethesda, Md.

The method of Rosenthal, Lillie and Verder (Fed. Proc. 11: 387, 1952) has been used to study the effects of various therapies in reducing the mortality occurring in burned mice during the 3 wks after injury. Mice were subjected to scald involving 2/3 of body surface and treated with 0.9% NaCl (15% B.W.) to prevent deaths from shock during the first 2 days. Serum and GG therapies were given in divided doses at 4-day intervals; antibiotics, daily for 5-14 days. Mortalities of the saline-treated (control) mice approximated 50% at 1 wk and 70% at 3 wks after injury. Chloramphenicol (CM) appeared to be the most effective in lowering mortality. Several other antibiotics were ineffective. CM, in doses as low as 5-10 mg/kg for 14 days, has given the most consistent therapeutic effect (reduced to 22% at 1 wk; 33% at 3 wks). Similar, but less marked, effects were observed when burned mice were treated with human GG or serum (CS) obtained from mice convalescing (3-8 wks) from burn injury. The effect from GG was observed only during the first wk; that from CS was prolonged through 3 wks. The initial response (at 1 wk) was essentially identical for GG and CS (10-20 ml/kg). The effect of combined (CS+CM) therapy was essentially additive.

13 *Pathology*

STUDIES ON EXPERIMENTAL SHOCK: PRODUCTION OF ISCHEMIC SKIN NECROSIS BY AN INTRADERMAL INJECTION OF ENDOTOXIN OR VASOPRESSOR AMINE. Carl G. Evers* and Joel G. Brunson. Path. Dept., Univ. Miss. Med. Center, Jackson, Miss.

Hybrid albino rabbits were subjected to a sublethal period of rotation (450 revolutions) in a modified Noble-Collip drum, in conjunction with an intradermal injection of endotoxin (100 mcgm.), or a vasopressor amine (epinephrine, levarterenal, phenylephrine, ephedrine, or isopropylarterenol). Areas of ischemic dermal necrosis developed at the site of injection in approximately 70% of the animals when the test substances were given simultaneously with or 4 hours after drum rotation, but in only 25% when they were given 4 hours prior to rotation. The lesions were characterized by extensive pale, flat ischemic areas which progressed rapidly to desiccation and subsequent desquamation. Histologically, there was ischemic epidermal necrosis with a paucity of inflammatory reaction except in the endotoxin lesions. Vascular occlusion was not observed. The lesions were prevented by administration of a mixture of promazine and promethazine, but were unaffected by prior administration of heparin. Pretreatment with nitrogen mustard increased the severity of the lesions. The results suggest that vasospasm, although playing an important role in causation of the lesions, is probably not the only factor involved.

14 *Immunology*

EXPERIMENTAL BURNS IN THE GERMFREE ANIMAL. Thomas G. Ward and Louise I. Lindholm.* Univ. of Notre Dame, Notre Dame, Ind.

To test the effect of bacteria on the shock mechanism in burns, the amount of heat expressed as LD_{50} in seconds was determined for germfree mice and for normal mice at various ages. The number of seconds required to kill half the animals when exposed by immersion in a 70°C water bath was significantly lower in the germfree mice. The amount of difference was inversely proportional to the age of the animals, germfree and normal, tested. Germfree and normal rats (Wistar) of comparable age and weight were bled, burned by a hot plate method, and sera collected at various intervals. The sera were tested for "HeLa growth preventive" factor. The normal rats responded more quickly and with a higher titer than the germfree. (Supported by a grant from Office of Naval Research.)

15 *Immunology*

THE COMPARATIVE PROTECTIVE ACTION OF HISTAMINE, L-HISTIDINE & THEIR METABOLITES ON ENDOTOXIN & THERMAL SHOCK IN MICE. Sigmund E. Lasker* & Charles L. Fox, Jr., Depts of Biochem. & Surg., N.Y. Medical College, Flower-Fifth Ave Hospitals.

While studying the relationship of lipopolysaccharides to shock [Fed.Proc. 1959, 18:87] we found that pretreatment with histamine was protective in endotoxin as well as thermal shock. Related substances were explored also. Protection against endotoxin (E.coli-Difco) was achieved by pretreatment with these metabolites; - imidazoleacetic acid, methylimidazoleacetic acid, the "histamine depletors" 48/80 and polymyxin, L-histidine and formiminoglutamic acid, the degradation product of urocanic acid. Not protective were acetylhistamine, D-histidine, urocanic acid (a metabolite of histidine), imidazolepropionic acid (its saturated analog), antihistimines, monamine oxidase inhibitors (aminoguanidine and Marsilid), diamine oxidase and histidine decarboxylase, Prednisolone was protective, and the protective action of cortisone, chlorpromazine, and serotonin was confirmed. In scald burns protection was achieved with those substances underlined above.

These results emphasize the importance of biochemical relationships in the shock syndrome.

Aided by the Defense Atomic Support Agency.

061. CARDIOVASCULAR SYSTEM—Blood Vessels

1 *Physiology*

LONGITUDINAL AND RADIAL STRAIN IN PULSE WAVE TRANSMISSION. Robert L. Van Citters and Robert F. Rushmer (intr. by Edward A. Boyden.) Dept. of Physiology & Biophysics, Univ. of Washington School of Med., Seattle, Wash.

Differences in the wave forms of pressure and dimensional pulses recorded at the same arterial site have been reported by many different investigators. To study this phenomenon further, aortic pressure, circumference or diameter, and length were recorded simultaneously. Pressure was recorded with a Sanborn differential pressure transducer via a short catheter, and circumference and length were recorded by means of delicate rubber tubing filled with mercury. Aortic diameter was recorded with mutual inductance coils. In general, the differences between the pressure and dimensional wave forms were diminished by improved recording techniques. Nevertheless, elongation of the ascending aorta preceded both elevation in pressure and expansion of the ascending aorta. This finding could be ascribed at least in part to abrupt descent of the aortic valve rings during isovolumetric contraction. Study of the propagation of pulses in large Penrose tubes showed that longitudinal waves traverse the wall at a velocity much higher than that of waves of either pressure or radial distention. Such longitudinal waves in the aorta might be of significance in altering the contour of the arterial pulse. (Supported by Cardiovascular Training Grant HTS 5147 from the National Heart Institute.)

2 *Physiology*

SOME EFFECTS OF SPLENECTOMY ON MESENTERIC BLOOD VESSELS AND THE SUSCEPTIBILITY TO HYPOTENSION AND SHOCK IN YOUNG ALBINO RATS. Leif Horn; (Intr. by M. Rockstein), Department of Pathology, New York University, College of Medicine, New York.

Studies in the past have shown that splenectomy affects several homeostatic systems, leading to changes in blood volume, blood coagulation, plasma proteolytic enzyme activity and edema formation. Microscopic observations on the mesocecum have shown that splenectomy in rats is followed by an increase in the number of capillary vessels with an active blood flow, a decrease in vasomotor activity, and a diminished sensitivity to topically applied epinephrine. It was further found that some of the effects of splenectomy may be aborted or counteracted by injection of a factor or factors extracted from the spleen. The role of the spleen in determining the susceptibility of the rat to hypotension and traumatic shock will be discussed on the basis of its effect on the microcirculation. It is further proposed that the spleen may influence vascular differentiation, and that it may be involved in regulating the sensitivity of the peripheral circulation to catecholamines. (Supported by a special fellowship U.S.P.H.S.)

3 *Physiology*

CUTANEOUS CAPILLARY TESTS: COMPARISON OF GRADED NEGATIVE PRESSURE, POSITIVE PRESSURE AND REACTIVE HYPEREMIA IN THE HUMAN. A. G. Lisi, S. Gollub and A. W. Ulin (intr. by J. R. DiPalma.) Blood Coagulation Research Laboratory, Hahnemann Hospital, Philadelphia, Pa.

The study of in vitro coagulation factors usually contributes little to our understanding of many cases of unexplained bleeding following surgery. As an approach to the study of possible vascular factors, a series of capillary tests were performed on the forearms of both surgical patients and controls. Positive brachial pressure was maintained for 5 mins. Graded negative pressures were applied on areas 5 mm. in diam. for 1 min. Reactive hyperemia was tested with a weighted ring left on the arm just long enough to first cause ischemia and then hyperemia (Stimulus Time), followed by a period of clearing (Reaction Time). (c.f. DiPalma et al, Am. Heart J. 23, 377, 1942.) A progressive response (in terms of petechial hemorrhages) with increase of negative pressure was noted for the majority of patients. Some correlation in reactivity was found between positive and negative pressure tests. In most subjects, the response to the Reactive Hyperemia test was more uniform as regards Stimulus Time while Reaction Time was more variable. There was no apparent correlation with clinical hemostatic abnormalities. (Aided in part by U. S. Army Grant DA-49-007-MD-720.)

4 *Physiology*

AORTIC PRESSURE-LENGTH-DIAMETER RELATIONSHIP. Dali J. Patel, Alexander J. Mallos*, Donald L. Fry, National Institutes of Health, Bethesda, Md.

It was the purpose of this study to examine simultaneous changes in length, diameter and intravascular pressure in living thoracotomized dog's aorta. Length and diameter were continuously measured by electrical calipers with adequate response characteristics. Preliminary results in 14 dogs include the following: (1) With inspiration the thoracic aorta increased in length and the abdominal aorta shortened. (2) During a cardiac cycle the thoracic aorta increased in length in phase with pulse pressure, ΔP (mean control value $+14 \times 10^{-3}$%/cm H_2O ΔP). The ratio of change in length to change in diameter varied between 5 to 26. (3) Longitudinal strain pulsations in abdominal aorta were usually 180° out of phase with ΔP. Similar inverted pulsations were also seen in the abdominal segment after complete occlusion of the aorta at the diaphragm. The longitudinal strain pulsations are thought to be produced by elongating strain of the thoracic aorta.

5 *Physiology*

REFLEX EFFECTS OF LEFT VENTRICULAR DISTENSION. C.E. Cross, P.F. Salisbury and P.A. Rieben (intr. by A. Surtshin) St. Joseph Hospital, Burbank, California.

In open-chest dogs on a heart-lung machine the right heart and the left atrium were drained and the pulmonary artery was obstructed. A balloon in the virtually bloodless left ventricle (LV) permitted its distension. LV and systemic arterial pressures (SAP) were recorded. The level of the calibrated venous reservoir of the heart-lung machine indicated increases or decreases of the dog's intracorporeal blood volume. Distension of the LV caused reversible changes of SAP and bradycardia (confirming Aviado and Schmidt) and also massive expansion of the intracorporeal blood volume, indicating dilatation of systemic veins. These effects of LV distension disappeared after interrupting conduction in the vagus. Conclusion: Sudden overdistension of the left ventricle to a diastolic LV pressure above 10-15 mm Hg resulted in bradycardia and dilatation of systemic arteries and veins. The observed effects of LV distension may operate as mechanisms which influence cardiogenic shock.

6 *Physiology*

ELECTROLYTES IN DOG CAROTID IN VITRO FOLLOWING ELECTRICAL AND EPINEPHRINE STIMULATION. V.E. Headings*, David F. Bohr, P.A. Rondell. Dept. of Physiology, Univ. of Michigan, Ann Arbor

The current study is concerned with a comparison of the effects of epinephrine and of electrical stimulation on the electrolyte composition of vascular smooth muscle. Rings of dog carotid were incubated for two to three hours in Krebs solution at 37° C. A contraction in response to epinephrine (10^{-6}) or electrical stimulation (5 volts) was recorded isometrically. Following stimulation a ring was removed from the Krebs solution for electrolyte analysis. The average potassium content per Kg tissue water for 16 electrically stimulated rings was 20 mEq less than the control value of 68 mEq derived from 29 unstimulated rings. The average sodium content of the electrically stimulated rings was 28 mEq greater than that of the unstimulated rings, which was 116 mEq. Both differences are statistically significant. Epinephrine, at a concentration giving a response as large as or larger than that resulting from the electrical stimulation, produced no significant change in electrolyte composition of the ring. These data indicate that there are basic differences between the components of the responses to electrical and to epinephrine stimulation. They also suggest that tension development by vascular smooth muscle stimulated by epinephrine may not be dependent on changes in sodium or potassium fluxes. (Supported by a grant from the Michigan Heart Association)

7 *Pharmacology*

EFFECT OF RESERPINE ON THE RESPONSE OF ISOLATED AORTA TO ELECTRICAL STIMULATION. Charles N. Gillis and Celia M. Yates.* Dept. Physiol. and Pharmacol. Univ. of Alberta, Edmonton, Canada.

The slow, secondary contraction of rabbit aortic spirals following cessation of an alternating current stimulus has been attributed (Furchgott, Fed. Proc.,11:217,1952) to the release of an epinephrine-like material (probably norepinephrine) from the artery. Pretreatment of animals with reserpine, which depletes the vessel wall norepinephrine (Burn and Rand, Brit.med.J.,1:903,1958), should therefore abolish the secondary contraction. On two successive days,rabbits received 1.5 mg/kg (i.p.) and 5.0 mg/kg (i.v.) reserpine. Spiral strips of aorta from these animals gave only the immediate rapid response during application of a 10V a/c stimulus. Norepinephrine (10-10), added to the bath fluid, caused a contraction of the tissue, but concentrations up to 10^{-7} did not restore the secondary response to electrical stimulation. It can be concluded that this part of the response is due to the liberation of norepinephrine and that the action of reserpine may be to prevent the uptake of the amine into the store postulated by Burn and Rand to be present in the vessel wall.

8 *Pathology*

EFFECTS OF HYPOGLYCEMIA ON THE BLOOD BRAIN BARRIER. Robert L. Abney*, Charles W. Hauser* and Joel G. Brunson. Path. Dept., Univ. Mississippi Med. Center, Jackson, Miss.

Previous studies (Am. J. Path. 34:631, 1958; Fed. Proc. 18: 480 and 499, 1959) described the effects of endotoxin, lowered serum calcium, heparin, and nitrogen mustard on the blood brain barrier. The present study was undertaken to determine the effects of hypoglycemia on the permeability of the cerebral vessels. Fifty hybrid rabbits weighing 1.5 kg. each were given an intravenous injection of crystalline insulin (80 units) or alloxan monohydrate (300 mgm./kg.). A state of shock developed within 5 to 10 hours following the injection, at which time the animals were given an injection of 2 ml. of a mixture of 2% trypan blue and colloidal iron into one of the carotid arteries. Control animals were given an intracarotid injection of trypan blue and colloidal iron mixture alone. The animals were sacrificed 15 to 30 minutes after administration of the colloidal substances. Of the experimental group, 36 (72%) showed gross staining of the brains by colloidal dyes, whereas only 6 of 29 controls (20%) evidenced such change. Microscopic examination disclosed the dye materials to be present in the arterial lumina and walls, and within the phagocytic cells of the central nervous system. The relationship of the development of these lesions to changes in serum proteins blood sugar and serum calcium and potassium will be discussed.

062. CARDIOVASCULAR SYSTEM—Blood Volume

1 *Physiology*

THE EFFECT OF A MASSIVE TRANSFUSION ON THE MOBILIZATION OR LOSS OF FLUID AFTER HEMORRHAGE. S. Deavers*, R. A. Huggins and E. L. Smith, Baylor U. College of Med., Houston, Texas.

Cell and plasma volumes and plasma proteins were measured by Cr^{51}, T-1824 dye and the falling drop method, respectively on morphine-pentobarbitalized dogs. After the control determinations an average of 86 cc/kg of blood was transfused, and 20 minutes later a series of measurements was done. The animals were then bled slowly through a femoral artery until the mean arterial pressure reached 35 mm Hg. When the dogs started to take the blood back another series of samples was drawn. Then the tube was opened and after 40% of the blood was taken back the remaining blood was pumped back, and after equilibration final determinations were made. Measured and expected red cell volumes were in good agreement throughout the study. However, 35 cc/kg of plasma was lost after the transfusion and an additional loss occurred after hemorrhage (8 cc/kg) and after reinfusion (7 cc/kg). Thus at the end of the experiment plasma volume approximated the control level. Changes in total proteins paralleled those of the plasma volume. The circulatory hematocrit reflected the changes in measured plasma volumes whereas the venous hematocrit did not.

2 *Physiology*

SIMULTANEOUS BLOOD VOLUME MEASUREMENT WITH Cr^{51} TAGGED CELLS AND T-1824. J. W. Remington and C. H. Baker, Dept. Physiology, Medical College of Georgia, Augusta.

When Cr^{51} tagged red cells are injected, the blood activity reaches a plateau value (stand. dev. of individual samples = 3%) within 20 min. After acute splenectomy, a plateau is reached in 10 min. When, in splenectomized dogs, repeated injections of tagged cells are made to measure the same cell volume (except for sample loss), the plateau levels may vary by 6%. After several such determinations, the cell volume is consistently greater than expected, just as with repeated injections of T-1824. When 14 dogs were bled step-wise, the tagged cells indicated too large a blood volume even after the 1st bleeding. The discrepancy became greater with further bleeding, or with more tag injection. The same trend was seen with T-1824. After severe hemorrhage, a blood volume based on a 10 min. concentration is definitely more discrepant than that had by a slope extrapolation. When bled dogs were re-infused, the Cr^{51} indicated too small a blood volume, but T-1824 did not. Some discrepancies in blood volume estimation seem referable to a change in body space through which the cells reach equilibrium, even in a time span of 70 min. (Supported by USPHS grant H-4573.)

3 *Physiology*

THE DISAPPEARANCE OF T-1824 FROM THE CIRCULATION OF NORMOVOLEMIC AND BLED DOGS. C. H. Baker and J. W. Remington. Medical College of Georgia, Augusta.

Time-concentration curves have been determined three successive times in 19 normovolemic dogs. In 24 dogs hemorrhaged in three steps, a control curve and one following each hemorrhage was obtained from a new injection of dye. There was no significant difference between the slopes of the three curves obtained from the normovolemic dogs when corrected for plasma specific gravity changes. However, the plasma volumes obtained from the second and third slopes averaged 10% higher than the control. This may be due, at least in part, to the wide variation in configuration of the slopes. In the bled dogs, the slopes of the curves became progressively steeper following each hemorrhage. The slopes of the control and second curves were questionably different, but the third was significantly steeper and the fourth much steeper. If no dye is injected following the last hemorrhage and the concentration of the previous injection is followed, a new and steeper slope occurs. Since the slopes are logarithmic, the loss of protein from the circulation apparently increases with the severity of the hemorrhage. Reduced lymphatic return along with anoxia of the capillaries increasing their permeability might be the explanation. (Supported by USPHS grant H-4573.)

4 *Physiology*

THE EVALUATION OF CHANGES IN BODY FLUID DISTRIBUTION BY REGIONAL WEIGHING. Colin Fell and Robert F. Rushmer (intr. by G. L. Maison.) Dept. of Physiology & Biophysics, Univ. of Wash. School of Med., Seattle, Wash.

A method for studying fluid shifts in experimental animals has been devised which involves simultaneously weighing various regions of the body. The anaesthetized animal is supported by electronic scales placed under the hindquarters, abdomen, thorax and head. Some interaction between the scales takes place, owing to coupling through the animal. A calibration factor which corrects for this effect is derived by using the algebraic sum of deflections of all the scales in response to a known weight. In a series of cats subjected to bleedings and infusions of known volumes, the total volume change appeared to be measured within a range of accuracy of 10-15 per cent. The partitioning between the hindquarters, abdomen and thorax varied, but the principal changes were seen in the abdomen and thorax. The method has also been used in a preliminary study of irreversible hemorrhagic shock in cats. Apart from the changes evident during the actual bleeding and reinfusion, no significant shifts in fluid distribution have been observed during the initial hypotensive period, or during the period following reinfusion of blood. (Supported by Cardiovascular Training Grant HTS 5147 from the National Heart Institute.)

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5 *Physiology*

RED CELL VOLUME IN THE KIDNEY OF THE RAT. P.N. Farnsworth (intr. by L.J. Cizek), Dept. of Physiology, Columbia University, New York.

Before the injection of red cells tagged with Cr^{51}, an abdominal incision was made and ligatures placed loosely around both kidneys in order that circulation in the kidney could be stopped without disturbing the abdominal viscera. The incision was closed to minimize the effects of changes due to an open abdomen. The red cell volume/gram of tissue in either kidney (0.072 ml/gm) is the same within an accuracy of 6% if they are tied off simultaneously or 10 min. apart; thus one kidney can serve as a control of the other. Red cell volume of a kidney was determined before and after a maximum hemorrhage either by using a syringe to forcibly withdraw blood or a free flow hemorrhage in normal animals and animals under Nembutal anesthesia (volume remaining, 0.036 ml/gm). The% of red cells removed (68%) was not found to differ significantly in either normal animals or anesthetized animals. This preliminary work is being followed by a study of the effects of adrenalin and the effects of varying degrees of hemorrhage on the kidney red cell volume. Adrenalin injection may decrease the red cell volume by 80% and a hemorrhage of 37% of red cells may decrease the red cell volume by 40%.

6 *Pharmacology*

SOME PHARMACOLOGICAL STUDIES OF A GLUTAMIC ACID-LYSINE COPOLYMER. M. Freeman Narrod. Children's Cancer Res. Fndn. and Dept. Pharmacology, Harvard Medical School, Boston, Mass.

These studies were undertaken as part of a long-term investigation of blood loss in leukemia and other disorders, conducted by Farber and his associates. The copolymer of glutamic acid and lysine (6:4) with an average molecular weight of 70,000 synthesized by Blout et al. (J. A. C. S., 80: 4909, 1958) has been examined and compared with dextran for its ability to expand the plasma volume of dogs, for some of its effects on the cardiovascular system of dogs, and for its effects in mice upon repeated administration. Doses of 40 ml/kg of a 3% solution of the copolymer expanded the plasma volume of 10 normovolemic and 15 hypovolemic dogs. This dose produced no change in the arterial blood pressure of normovolemic dogs. Average doses of 450 ml of a 3% solution of the copolymer in the dog heart-lung preparations produced no changes in heart rate, right atrial blood pressure or in the ability of the heart to respond to an increased work load. No deleterious effects were seen in mice when the copolymer (40 mg/kg of a 3% solution) was administered intravenously either once a week or biweekly for 16 weeks.

063. CARDIOVASCULAR SYSTEM—Cardiac Dynamics

1 *Physiology*

RELATIVE MITRAL INSUFFICIENCY AS A FACTOR OF PAROXYSMAL PULMONARY EDEMA. Aldo A. Luisada, Aldo Jacono, and Marvin Kaplan.* The Chicago Medical School, Chicago, Ill.

The pressure curves from the pulmonary artery, left atrium, and left ventricle (or aorta) were studied in 17 dogs following stimulation of the CNS through intracisternal injection of veratrine. The systolic pressures of the aorta and left ventricle rose to extremely high levels. The diastolic pressures of the left ventricle rose in all experiments where pulmonary edema developed. However, the level of the diastolic pressure was often not sufficiently raised for causing edema. A typical plateau pattern was observed in the left atrium of all dogs which developed pulmonary edema. This systolic plateau, interpreted as the result of "relative" mitral insufficiency, further raised the mean pressure of the left atrium (and of the pulmonary vessels) above the threshold of transudation. It is postulated that this valvular insufficiency was caused by extreme dilatation of the left ventricle and represented an important factor in the mechanism of pulmonary edema. (Supported by Grant H-3362 of the National Heart Institute, U.S.P.H.S.)

2 *Physiology*

INSTANTANEOUS DIMENSIONAL CHANGES OF THE LEFT VENTRICLE IN AWAKE DOGS. E.W. Hawthorne, M. Gaspar* and W. G. Pogue*. Howard Univ. Med. School, Wash. D.C.

In awake, intact dogs the cardiac cycle starts with an isovolumic period in which there is shortening of the base to apex length with an increase in cross sectional area. Total shortening is about 1/4 of that in "open chest" dogs. Minimal changes in base to apex length occur during the ejection phase and there is a typical pattern of decrease in cross sectional area. During isovolumic relaxation, the left ventricular base to apex length increases. Rapid filling is characterized by a sudden increase in cross sectional area and is followed by a diastasis of varying lengths. No significant changes in cross sectional area and length are seen in this phase. Atrial systole is associated with a sudden increase in cross sectional area and a decrease in base to apex length. Our studies suggest that the ventricle contracts in an "eccentric manner" and relaxes in a "concentric fashion". The methods used, typical recordings of the above, and other findings will be presented. Care must be exercised in deriving information concerning volume and fiber length from a single dimensional measurement of the left ventricle at most points during the cardiac cycle.

3 *Physiology*

ON TAKING THE FIRST DERIVATIVE OF THE PULSE, AND ITS RELATION TO THE BALLISTOCARDIOGRAM. Isaac Starr and S. Ogawa*. University of Pennsylvania, Philadelphia, Pennsylvania.

Using a differentiating electrical circuit, adapted to our purpose by Mr. George Pierce and constructed by Mr. Lee Gorden, an "Infraton" pulse transducer and two Sanborn Twin Viso recorders, we have secured simultaneous records of the EKG, brachial pulse, the 1st derivative of that pulse, and the ballistocardiogram. Tests of accuracy made by insertion of sine waves, square waves and triangular waves at varying frequencies show that our record of the pulse derivative lags a bit at high frequencies, but it seems satisfactory enough for the main frequencies found in the pulse wave. The well-known disadvantages of pulse records taken by any transducer on the skin over an artery apply also to records of the derivative, but the latter have certain advantages over conventional records, expecially in the greater stability of the base line. The derivative emphasizes certain aspects of the pulse wave related to the higher dynamic functions of the heart, which are recognized with difficulty, if at all, in the classic pulse wave. The second derivative of the pulse, calculated from the first graphically, often resembles the ballistocardiogram quite closely, as was pointed out by Reeves, et al. But in some cases the resemblence is not close. (Supported by a grant from U.S.P.H.S. - H-625 C-10)

4 *Physiology*

THE BALLISTOCARDIOGRAPHIC RESPONSE TO HYPERTHERMIA. Arthur J. Moss and Bruce E. Bradley (intr. by A. Graybiel.) U.S. Naval School of Aviation Medicine, Pensacola, Fla.

The body temperature of five normal subjects was raised to levels above 100.0°F. through the use of a rubberized anti-exposure suit. Immediately after removal from the anti-exposure suit the induced hyperkinetic cardiovascular state was studied on an ultra-low-frequency acceleration ballistocardiograph. The ejection deflection (HI wave) consistently increased in amplitude and became more vertical during the hyperthermic state. The HJ amplitude showed changes comparable to the alterations seen in the HI amplitude, but to a lesser degree. The time duration from the onset of ventricular ejection to the J wave peak generally was shortened. Twenty minutes after removal from the anti-exposure suit the body temperature and the ballistocardiographic measurements returned to the normal values obtained prior to the induced hyperthermia. Diminished peripheral resistance and its sequelae seemed to play a major role in the ballistocardiographic changes seen with hyperthermia.

Physiology

5

EVALUATION OF "VENOUS RETURN" INITIATING INCREASED CARDIAC OUTPUT. Robert F. Rushmer, Don Baker* and Robert L. Van Citters*. Dept. of Physiology & Biophysics, Univ. of Washington School of Med., Seattle, Wash.

Increased cardiac output at the onset of exertion is often ascribed to increased "venous return" induced by the pumping action of contracting skeletal muscles. Changes in left ventricular performance closely resembling those seen during exertion are commonly observed without evident motion by intact unanesthetized dogs; i. e. during "anticipation" of exertion, eating or viewing a plate of dog food or electrical stimulation of discrete sites in the diencephalon. To investigate the role of increased "venous return" in initiating increased cardiac output during exercise, ultrasonic flowmeters were installed on the inferior vena cava and on the ascending aorta to learn if increased inflow into the right ventricle significantly preceded the increased outflow in the aorta. The transition at the beginning of exercise was somewhat obscured by fluctuations in vena caval flow patterns (possibly respiratory), but outflow from the left ventricle increased almost simultaneously or within 2-3 seconds after the inflow into the right ventricle increased. (Supported by grants from the American Heart Association and the National Institutes of Health.)

Physiology

6

MYOCARDIAL CONTRACTILITY UNDER HYPOTHERMIA. E. T. Angelakos and A. H. Hegnauer. Dept. of Physiology, Boston Univ. School of Medicine, Boston, Mass.

Left ventricular contractility was evaluated in dogs subjected to immersion hypothermia from measurements of myocardial tensions and intraventricular pressures. Myocardial contractility is decreased during hypothermia as manifested by decreased peak tension and decreased rate of isometric contraction and relaxation. The myocardial tension curves were considered as statistical distributions and were analysed to obtain the parameters of mean, mode, median and variance corresponding to each curve. Comparison of these parameters as they are affected by hypothermia suggests that the negative inotropic effects of cold in the intact heart are associated with increased asynchronism of left ventricular contraction. This is probably due in part to the decreased conduction velocity produced by hypothermia. In general the hypothermic heart appears to be in a state of incipient failure; although the cardiac performance is adequate for the demands of the hypothermic condition, the cardiac reserve is reduced thus similating moderate cardiac failure. Thus the hypothermic heart may serve as an experimental model of reversible heart failure. (Supported by USPHS grant H-4355).

Physiology

7

DETERMINANTS OF THE RELATIONSHIP BETWEEN LEFT VENTRICULAR END DIASTOLIC PRESSURE AND CIRCUMFERENCE. Eugene Braunwald, Robert L. Frye*, and John Ross, Jr.*. National Heart Institute, Bethesda, Md.

The relationship between left ventricular end diastolic pressure (EDP), and circumference (EDC), measured with a mercury resistance gauge, was studied in 14 dogs with open chests. In 6 experiments utilizing a preparation described previously (Am. J. Physiol. 192:157, 1958), aortic pressure, cardiac output, and heart rate were controlled and varied independently. Acute heart failure, evidenced by depressed ventricular function, resulted in a larger EDC at any given EDP. Acetylstrophanthidin augmented stroke work at any EDC, and thus increased systolic emptying. In 6 of 7 dogs this drug did not modify the relationship between EDP and EDC. In 5 dogs hypothermia diminished EDC at any EDP. In 6 dogs in which cardiac output was progressively increased at various aortic pressures, the relationship between EDP and EDC remained constant. There was no consistent relationship between EDC or EDP and the Tension-Time Index, the primary hemodynamic determinant of myocardial $\dot{V}O_2$ (Am. J. Physiol. 192:148, 1958). This data is therefore not consonant with the view that myocardial $\dot{V}O_2$ is primarily dependent upon end-diastolic fiber length.

Physiology

8

THE EFFECT OF ATRIAL FIBRILLATION ON THE VOLUME-PRESSURE RELATIONSHIPS OF THE LEFT HEART. Robert C. Little. Dept. of Physiology, Seton Hall College of Medicine and Dentistry, Jersey City, New Jersey.

Volume-pressure relationships were determined in the living dog for the left heart-pulmonary vascular segment with the atria relaxed and again during atrial fibrillation. The pulmonary-left heart segment was isolated by occluding the pulmonary artery during brief periods of ventricular standstill. During this interval of cardiac arrest a measured volume of saline was injected into the left atrium and the change in pressure recorded. (Fed. Proc. 18:95, 1959). Atrial fibrillation was produced by electrical stimulation of the atria. Volume-pressure curves for each animal were constructed by plotting the change in left atrial pressure against the volume injected. Atrial fibrillation did not change the slope of the curve, however, the entire curve was displaced toward the volume axis. With any change in volume, the change in pressure was in the order of 1 mmHg lower in the fibrillating heart. The explanation for this shift is not clear but it may represent a change with fibrillation in the geometry of the atrium or in the "tonus" of the atrial myocardium. (Supported in part by the American Heart Association).

Physiology

9

RIGHT VENTRICULAR VOLUME AND PRESSURE IN DOGS, HORSES, CATTLE AND SWINE. E. A. Rhode*, J. P. Holt, H. Kines* and S. A. Peoples. Heart Laboratory, Univ. of Louisville and Univ. of California School of Veterinary Medicine.

In anesthetized closed-chest dogs, horses, cattle and swine subjected to plethora and hemorrhage rt. ventricular end-diastolic pressure (EDP) and end-systolic pressure (ESP) were measured, and end-diastolic volume (EDV), end-systolic volume (ESV), and stroke volume determined by means of an electric conductivity method described earlier by one of us (J.P.H.). In animals in the control state, ranging in size from 18.5 to 836 Kg., there was an approximately linear relationship between EDV and ESV, (ESV = 0.53 EDV), and between EDV and stroke volume, (S = 0.47 EDV); and a curvilinear relationship between cardiac output (CO) and EDV, ($CO = 118\ EDV^{.75}$), and between heart rate (R) and EDV, ($R = 250 + EDV^{.25}$). In all animals studied a several fold increase in EDV, from the minimum value consistent with life, was associated with little or no change in the EDP; while a small increase in ESV, from the minimum value consistent with life, was associated with a steep rise in ESP to a certain level, while further increase in ESV was associated with little or no increase in ESP. (Supported by USPHS Grants #1534 and #2075; The Ky., Louisville, and Amer. Heart Assns.)

Physiology

10

EFFECT OF ETHYL ALCOHOL INFUSION ON CORONARY AND SPLANCHNIC HEMODYNAMICS. Paul W. Willard* and Steven M. Horvath. Lankenau Hospital and Jefferson Medical College, Philadelphia, Pennsylvania.

Discrepancies have arisen as to the effect of Ethanol on the coronary and hepatic circulation. To more clearly define the parameters, coronary and hepatic blood flows were measured in 14 mongrel dogs (17.7 kg. body wt.) before, during, and following ethyl alcohol infusion. A slow intravenous administration of 500 mg. of 95% alcohol was given over a 60 min. interval. No significant change in coronary or hepatic blood flow was noted at any point during the procedure. The various resistances over the entire vascular bed showed no change. However, a significant decrease in left ventricular work was noted during and following the infusion. The arterial concentration of alcohol increased during and decreased after the infusion in an exponential manner. There was a decreasing extraction of alcohol by the liver concurrent to the increasing concentration of arterial alcohol. The interrelationship of blood alcohol concentration to cardiac and splanchnic hemodynamics and metabolism is dependent upon dose and rate of admin.

1 *Physiology*

THE EFFECT OF STELLATE GANGLION STIMULATION ON THE PRODUCTION OF CATECHOLS BY THE HEART. J.H.Siegel*, J.P.Gilmore, S.J.Sarnoff, (intr. by Dr.J.Shannon) Lab. Cardiovascular Physiol., NHI, Bethesda, Md.

An experimental canine preparation was employed in which the total vena caval return to the heart was diverted into a reservoir, and then pumped into the main pulmonary artery at a constant predetermined rate so as to bypass the right heart. The right ventricle was allowed to eject the entire coronary venous outflow, via a cannula, into a recording rotameter circuit from which it was returned to the venous reservoir. The analyses of arterial and coronary venous blood, by a modification of the fluorometric technique of Weil-Malherbe and Bone, indicated that stellate ganglion stimulation is accompanied by an increased coronary venous concentration of norepinephrine. This increase is associated with hemodynamic evidence of a positive inotropic effect and is independent of increases in coronary flow, heart rate, or stroke volume. A finding of interest was that cessation of stellate stimulation was frequently followed by the appearance in coronary venous blood of large quantities of a substance which gives a positive fluorescent peak similar to that for norepinephrine but which was not accompanied by any demonstrable hemodynamic effects.

2 *Biochemistry*

WATER AND ELECTROLYTE STUDIES ON CANINE HOMOTRANSPLANTED HEARTS. Peter V. Moulder* and Lillian Eichelberger. Department of Surgery, The University of Chicago, Chicago.

Biochemical structure of the myocardium defined in terms of extracellular and intracellular fluid and solid phases and electrolyte concentrations in these phases has been defined in six canine hearts homotransplanted to the necks of donor animals and allowed to remain 3 to 72 hours.

(1) Within these time limits, there seems to be no progressive pattern.
(2) For a number of hours (at least six) this biochemical character of the transplanted heart can remain within normal limits.
(3) In some early transplanted hearts and in all prolonged transplanted hearts, there is a marked extracellular edema and this is sometimes associated with some intracellular dehydration.
(4) Despite the water changes there is a remarkable tendency for intracellular concentration of the cations potassium and magnesium to remain near normal.
(5) Sodium partitions remain normal.
(6) There is an inconstant and widely variable increase in the amount of calcium in the heart. $(Ca)_m$ is normally 1.93 mEq/Kg and has been found as high as 6.70 mEq/Kg in some experiments.

(Aided by a Grant-in-Aid from American Heart Association.)

12 *Physiology*

A PHYSIOLOGIC EXPLANATION OF ABNORMAL ARTERIAL BLOOD PRESSURE RESPONSES TO THE VALSALVA MANEUVER. Ross C. Kory, John O. Chamberlain* and Howard L. Correll.* Wood V.A. Hospital and Marquette Univ. School of Med., Milwaukee, Wis.

Consistent arterial blood pressure responses to the Valsalva maneuver have been described for normal persons and for patients with congestive heart failure, mitral stenosis, and pulmonary emphysema. The "congestive" pattern of response has been ascribed by some to right ventricular failure alone. Others have related this pattern to either right or left heart failure. Valsalva maneuvers with direct brachial artery pressure recording were performed in 10 normal subjects and 47 patients with cardiac or pulmonary disease. An intra-oral pressure of 40 mm. Hg was maintained for 10 seconds for each test. Right heart catheterizations were performed in 12 of the patients. The "congestive" response was always related to pulmonary vascular congestion whether or not right heart failure was present. Right heart failure alone was never associated with a "congestive" response. Patients with pulmonary emphysema consistently demonstrated an exaggerated form of the normal response. A physiologic explanation is advanced which relates the various normal and abnormal Valsalva responses to the central blood volume, i.e. to the ratio:

$$\frac{\text{Cardiac output}_{10\text{ sec.(period of the test)}}}{\text{Central blood volume}}$$

3 *Biochemistry*

EXCRETION OF GLUTAMIC-OXALACETIC TRANSAMINASE IN THE BILE OF RATS. R. M. Hill, Barbara Nelson*, and D. D. Sowl*, University of Colorado School of Medicine, Denver, Colorado.

Glutamic-oxalacetic transaminase is released into the blood stream following tissue damage in any part of the body. Whatever the level reached in the plasma the enzyme rapidly disappears. Its fate is unknown. Little or none appears in the urine. In the work reported here, excretion in the bile of rats was measured over periods up to 25 hours following I.V. injection of transaminase into normal and B_6 deficient adult rats. Both myocardial extracts containing known amounts of transaminase and solutions of purified apotransaminase were used. After I.V. injection of normal saline (controls), both normal and B_6 deficient rats excreted very small amounts of transaminase in the bile intermittently, the total amount never being more than 80 units in 10 hours. None of the rats excreted in the bile more than 1% of the injected transaminase or apotransaminase. When purified apotransaminase was injected the ratio of holo- to apotransaminase in the bile was 1:2. The ratio of holo- to apotransaminase in the myocardial extract administered was 2:1. After injection of this extract, the ratio of holo- to apotransaminase in the bile was 4:1. The total transaminase excreted in the bile by B_6 deficient rats after I.V. injection of apotransaminase was comparable to that excreted by normal rats but the ratio of holo- to apotransaminase in the bile was only 1:6. (Grant from American Heart Association.)

064. CARDIOVASCULAR SYSTEM–Cardiac Muscle

1 *Physiology*

POSSIBILITY OF JUNCTIONAL TRANSMISSION IN CARDIAC MUSCLE. Nick Sperelakis and T. Hoshiko (intr. by D. B. Hackel.) Physiology Dept., Western Reserve Univ., Cleveland, Ohio.

Recent work demonstrating that the myocardium is neither a morphological nor functional syncytium suggests spread of excitation by junctional transmission between cells. The resistance of one myocardial cell was about 12 megohms while that between two cells was double, indicating the cells are functionally small units with no low resistance pathways between cells. Therefore, the rising phase of the action potential was studied for evidence of junctional potentials. Three components were identified in the rising phase: a slowly rising foot, a rapidly rising middle portion and a final slow rise to the peak. With partial withdrawals the rapidly rising mid-portion became gradually converted into a momentary reversal of the potential forming a prominent notch. The slowly rising foot up to the notch may or may not be due to a junctional process. However, a pronounced "prepotential" or "foot" in isolation alternated with a prepotential plus action potential response under conditions of possible junctional fatigue in cat Purkinje fiber and in frog ventricle. Some evidence has been obtained for different propagation velocities in opposite directions along a frog ventricular strip. These results are consistent with the junctional transmission hypothesis.

4 *Pharmacology*

THE INFLUENCE OF SYMPATHETIC ACTIVITY ON PHOSPHORYLASE IN THE NON-PERFUSED RAT HEART. Marilyn E. Hess*, Joseph Shanfeld*, Niels Haugaard. Univ. of Penna., Schools of Med.,Philadelphia, Penna.

Non-perfused hearts contain much lower amounts of phosphorylase a than perfused hearts. In the perfused heart, sympathetic stimulation is absent and norepinephrine content is decreased. A study in the intact rat of the influence of changes in sympathetic activity on cardiac phosphorylase a was made. Ether, pentobarbital, Reserpine, adrenalectomy, bretylium with and without adrenalectomy were used to decrease sympathetic stimulation and/or tissue content of catecholamines. The hearts were immediately frozen in alcohol-dry ice. Phosphorylase determinations were made after treatment of the extract with Dowex 1-X4 anion exchange resin to remove AMP. Each of the procedures used decreases sympathetic stimulation and/or release of catecholamines by a different mechanism. The results obtained with bretylium, adrenalectomy, and bretylium plus adrenalectomy show that release of catecholamines from the adrenal medulla plays a minor role compared with sympathetic stimulation in influencing the amount of phosphorylase a in the heart. Reserpine also effectively decreases phosphorylase a levels. These results suggest an intimate relationship between the activity of the sympathetic nervous system and phosphorylase a in the heart.

5 *Pharmacology*

ALDOSTERONE, LANATOSIDE C AND ISOLATED RAT HEART. George Sayers and Neil Solomon.* Department of Physiology, Western Reserve University, Cleveland, Ohio.

The isolated rat heart was perfused with oxygenated 0.9% NaCl solution (recirculated) (32° C) via a pulmonary vein and operated at a mean arterial pressure of 80 mm Hg. Four experimental groups were compared: 1) Control (no addition) 2) Aldosterone (0.9% NaCl plus aldosterone, 3.2×10^{-2} µg per 100 ml), 3) (0.9% NaCl plus Lanatoside C 0.1 µg per 100 ml), 4) Tetrahydrocortisol (0.9% NaCl plus THF 3.2×10^{-2} µg). The table presents, No. (number of preparations); DF (duration of function in minutes over which isolated heart expelled perfusate at 80 mm Hg); ICO (initial cardiac output in ml/min., first recording of cardiac output after stabilization and which includes coronary flow); LVWI (left ventricular work index in gram-Meters calculated as product of sum of cardiac outputs of successive intervals and mean arterial pressure).

Group	No.	DF	ICO	LVWI
1. Control	(10)	6	11.1	31 ± 6
2. Aldosterone	(10)	29	11.6	249 ± 43
3. Lanatoside C	(4)	26	11.3	223 ± 19
4. THF	(10)	7	11.4	40 ± 7

Aldosterone and Lanatoside C at low concentrations prolong the function of the isolated rat heart perfused with 0.9% NaCl.

6 *Pathology*

AN UNUSUAL SPONTANEOUS CARDIAC LESION OF UNKNOWN ETIOLOGY IN MICE. R. J. M. Fry*, K. Hamilton* and H. Lisco. Argonne National Laboratory, Lemont, Illinois.

An enlarged and hemorrhagic left auricle filled with thrombi was found in a considerable proportion of inactive female breeders in BALB/C mice from 300 to 700 days old. This lesion, which so far has been observed only in its advanced stages, was characterized by old focal areas of necrosis of the myocardium with calcification, extensive loss of muscle fibres and variable degrees of fibrous replacement which often contained islands of hyaline cartilage. The fibrosis was frequently associated with an unusual degree of vascularity. The auricles were filled with one or more large thrombi in various stages of organization and they were usually dilated to 3-5 times their normal size. The condition was restricted to the left auricle and no changes have been found elsewhere in the myocardium or vascular system. The etiology and pathogenesis of the condition is obscure, although the possibility has been considered that repeated pregnancies may be of some significance. (Work performed under the auspices of the U. S. Atomic Energy Commission)

065. CARDIOVASCULAR SYSTEM—Cardiac Muscle—Contraction

1 *Physiology*

RELATION OF WORK AND OXYGEN CONSUMPTION IN ISOLATED CAT MYOCARDIUM. William J. Whalen[1] (intr. by V. E. Hall) Univ. Calif. School of Medicine, Los Angeles, California.

Our previous studies indicated that the QO_2 of isolated mammalian myocardium was determined by initial length and frequency of contraction rather than by isometric tension developed. To ascertain whether work might be a significant factor, the muscle was allowed to shorten and to lift a weight. A wide range of loads and initial lengths was used. In alternate periods the contractions were made isometric, shortening being less than 3%. With the bath temperature at 31.7°C and the muscle stimulated at 30 per min., the amount of shortening, the developed isometric tension and the oxygen consumption were recorded. Data from 32 cat papillary muscles and 14 cat trabeculae carnae revealed no significant increase in QO_2 as the result of the work. It would appear that for any given muscle length a "package" of energy is released with each contraction. The amount of energy set free is apparently independent of load or shortening. Thus, isolated cardiac muscle may differ fundamentally from isolated frog skeletal muscle which shows increased energy liberation with work. (Supported by grant H-3201 from the National Heart Institute, Public Health Service.)

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2 *Physiology*

THE EFFECTS OF STRETCH ON TENSION DEVELOPMENT IN THE ISOLATED FROG HEART. Arthur J. Kahn (intr. by Ernesto D. Salgado.) Dept. of Physiology, Seton Hall College of Med. and Dent., Jersey City, New Jersey.

It has been reported that isometric contractile tension of frog atria and ventricles (Kahn, A.J., Fed. Proc. 18:78, 1959) and of papillary muscle (Kelly, J.J.and Hoffmann, B.F., Fed. Proc. 17:85, 1958) remains at peak levels over a wide range of initial tensions greater than that required to reach the maximum. Examination of the effects of stretch on several other parameters in isometrically contracting hearts has shown characteristic changes. The maximum rate of tension development follows a curve which shows an optimal initial tension and a shape suggestive of the "Starling curve". A curve of similar shape is obtained for the maximum rate of negative tension change during the relaxation phase of these responses even though unaccompanied by any decrease in the peak tensions developed in the contraction. It appears that stretch, in a range beyond that necessary to produce a maximal peak isometric output in these hearts, does not alter the peak tension, but significant changes in the rate of contraction and relaxation of at least some of the muscle fibers does occur. This may in turn account for the decompensatory phase of the myocardial response in failure. (Supported by USPHS Grant # H-4738).

3 *Physiology*

MEMBRANE DEPOLARIZATION AND CONTRACTION IN AURICLE. Frederic Kavaler (intr. by E.E. Suckling) State University of New York, Downstate Medical Center, Brooklyn, New York.

Using a method previously described (Am. J. Physiol, 197:968,1959), the time courses of transcellular membrane potential and of mechanical tension were simultaneously recorded from small (0.5-1.0 mm in diameter) muscle bundles taken from the right auricle of the dog. Tension was recorded, as before, over a short interval of length (0.3mm) of the muscle. Over this length the fiber membrane could be kept depolarized, by current flow, for a period several times the duration of a normal action potential. The tension time course during maintained depolarization (2 sec) differed very little from that accompanying a normal action potential (duration 80 msec). An initial rise and fall in the tension record duplicated the normal twitch. The subsequent time course during maintained depolarization showed some small, slow return of tension, which then subsided on repolarization. In the absence of any prolonged contractile response to maintained membrane depolarization, the behavior of auricle is in marked contrast to that of ventricle.

4 *Physiology*

INFLUENCE OF CORONARY ARTERY PRESSURE UPON THE DISTENSIBILITY OF THE CANINE LEFT VENTRICLE. P.F.Salisbury, C.E.Cross* and P.A.Rieben*. St. Joseph Hospital, Burbank, California.

In open-chest dogs with extracorporeal circulation the right heart and the left atrium were drained. A catheter tip at the coronary ostia communicated through the left subclavian artery with a perfusion reservoir. The ascending aorta was tied on the catheter, separating systemic and coronary circulations. Pressures were registered in the left ventricle (LVP), a systemic artery (SAP), the coronary perfusion system (CP). A balloon in the left ventricle permitted its distension. A pacemaker regulated heart rate. The mitral valve was occluded and the LV outflow tract was closed by a subcoronary clamp or ligature. The hydraulic isolation of the LV was tested under direct vision at the end of each experiment. LVdiastolicP was decreased by lowering CP and increased by raising CP; SAP had no influence upon LVdP in this type of experiment. Conclusion: Elasticity (distensibility) of stretched LV myocardium was influenced by coronary artery pressure. This is interpreted as indicating an erectile function of the coronary tree.

XUM

5 **Physiology**

DEPRESSION OF TENSION BY CURRENT APPLIED DURING THE ABSOLUTE REFRACTORY PERIOD OF HEART MUSCLE. Allan J. Brady (intr. by John Field). Univ. of California, Los Angeles.

Current pulses applied to strips of heart muscle during the absolute refractory period reduce the shortening velocity and peak tension of the twitch during which the pulses are applied. Depression is directly related to total charge, i.e. current x time, applied to the tissue. This phenomenon can be explained by a propagated repolarization through part of the tissue, induced by the applied currents. At the termination of single current pulses tension rises at a near normal rate suggesting anodal break excitation of the repolarized fibers. Long initial excitatory current pulses reduce the shortening velocity presumably by temporary hyperpolarization of part of the fiber membrane. These effects are being explored because of the evidence they supply regarding the connection between membrane processes and contractility.(From the Los Angeles County Heart Association Cardiovascular Research Laboratory in the Department of Medicine)

6 **Physiology**

ARRHYTHMIA AS A DETERMINANT OF VENTRICULAR CONTRACTILE FORCE. B. Lendrum*, H. Feinberg and L.N. Katz. Med. Res. Inst., Michael Reese Hosp. & Med. Center, and Ped. Cardio. Physiol. Dept., Cook County Child. Hosp., Chicago, Ill.

The left ventricular pressure pulse, developed at constant vol. in the in situ dog heart, was: 1) increased with heart rate regardless of pacemaker site, heart vol., and art. O_2 sat. 2) decreased in premature beats regardless of origin; more so, the greater the prematurity. 3) greatly increased in the post-extrasystolic beat and with decrement in several succeeding beats; occasionally, potentiation was greatest in the second beat; several successive premature beats increased the potentiation. Heart rate and premature beat effects were additive. Post-extrasystolic potentiation was superimposed upon alternans, when present. Therefore, the timing of the heart beat alters its contractile force independent of the well-known effect of diastolic fibre length (ventricular filling).

7 **Physiology**

PARADOXICAL RESPONSES OF THE MURAL STRAIN GAUGE ARCH TO LOCALIZED ISCHEMIA. A.H. Katcher, M.E. Hess, G. Peirce, J.J. Sayen, W.F. Sheldon, and P. Crum. (intr. by O Horwitz).

Contraction of local areas of the myocardium of dogs was studied by measuring the change in distance between multiple points on the left ventricular surface from projections of motion picture film (single and stereoscopic views at 64 frames/sec). Normal contraction showed considerable variation in amount, timing, and duration. Occlusion of a coronary artery branch was followed within 10 sec. by bulging of the ischemic surface in late systole. The bulge was holosystolic in 60-80 sec. The strain gauge failed to reflect normal local variation in tension because its application necessitated stretching the muscle beneath it. When the gauge was applied in the conventional manner with high degrees of stretch there was no response to ischemia or only a slow increase in amplitude and decrease in width of the positive systolic deflection. With less stretch the gauge could mimic surface contraction with a late systolic negative deflection appearing within 10 sec. and proceeding to holosystolic negative deflections in 60 sec. Neither type of response corresponded to the increase in tension within the bulge predicted from the unaltered intraventricular pressure. Interpretation of the strain gauge response to local ischemia requires prior knowledge of surface contraction. (Supported by USP grants HF-7812 and H-398.)

8 **Physiology**

EFFECTS OF HYPOPHYSECTOMY ON MYOCARDIAL FUNCTION AND COMPOSITION. W.V.Whitehorn, T.M.King*, and B. Reeves* Dept. of Physiol., U.of Ill., Chicago.

Previous observations of role of gonadal steroids in regulation of myocardial function indicated reduction of contractility of surviving and glycerinated columnae carneae of ovariectomized rats. Ventricular actomyosin concentration was proportionately reduced. Studies were extended to define possible role of hypophyseal factors. Maximum developed tension of surviving columnae of 8 hypophysectomized females was not different from that of 10 controls. Heart-body weight ratios were reduced. Ventricular total protein and actomyosin concentration were not modified by hypophysectomy. However, extracted contractile protein of experimental group showed reduced viscosity drop on addition of ATP. Results indicate significant differences between effects of castration and hypophysectomy on myocardial function and composition and suggest possible change in nature of contractile protein in the latter. Importance of pituitary-gonadal interplay in regulation of cardiac contractile system is indicated. (Supported by Grant H-1353, NHI)

9 **Biochemistry**

CREATINE, CREATININE, AND CREATINE PHOSPHATE IN HEART HYPERTROPHY. E.C.Gangloff*, I.L.Hemmings*, J.W.Wotring, Jr., J.S.Griffith*, and R.F.Krause. Department of Biochemistry, West Virginia University Medical Center.

Exercise, and hypoxia are known to produce heart hypertrophy. To compare the chemical changes in this process, an experimental and a control group of male adult Wistar strain rats were used for each stimulus: (I) running for 2 hours/day at one mile/hour for a total of 64 hours; (II) hypoxia in an altitude chamber, 24,000 feet, 8 hours/day for a total of 250 hours. The heart-body weight ratios observed were respectively 2.95, 2.90; (I) 3.41; (II) 4.21. Creatine and creatine phosphate were separated in a cold room on Dowex 50, and the fractions measured as methyl guanidine by the technique of Van Pilsum et al (J.B.C. 222 225, (1956)). No significant differences in creatine and creatinine levels were found in hypertrophies (I) and (II). The creatine present as creatine phosphate was increased in (I) and (II) 40-70%. It is concluded that a similar chemical storage of energy is induced in each of the heart hypertrophy-producing situations studied.

(Supported by a grant from West Virginia Heart Association)

10 **Pharmacology**

COMPARISON OF THE INOTROPIC EFFECTS OF CERTAIN PYRIDINE COMPOUNDS AND THEIR CORRESPONDING N-OXIDES. Hollis G. Schoepke* and Frederick E. Shideman. Dept. of Pharmacology and Cardiopulmonary Research Laboratory, Univ. of Wisconsin, Madison.

The inotropic effects of pyridine, 2-aminopyridine and 2-, 3- and 4-methyl- and 2,6-dimethylpyridine and their corresponding N-oxides have been studied on the isolated papillary muscle of the cat. Pyridine (5-20 mM) produced small negative inotropic effects. A positive inotropic effect generally preceded by a small, transient, negative inotropic effect was seen with higher concentrations. The methylpyridines (0.5-2 mM) produced positive inotropic effects which, at higher concentrations, were preceded by a small transient negative inotropic effect. Positive inotropic potency increased with increasing methylation. 2-Aminopyridine exhibited only positive inotropic activity. The positive inotropic effects of the above compounds were blocked by dichloroisoproterenol (DCI), trimethyl-[2-(2,6-xylyloxy)-propyl]-ammonium chloride (SKF 6890-A) and reserpine suggesting that they produce this effect by a catecholamine release mechanism. The N-oxides provoked only positive inotropic responses and were less potent on a molar basis than the corresponding pyridine compounds. The positive inotropic effects of the N-oxide compounds were not blocked by DCI, SKF 6890-A or reserpine suggesting a different mechanism of action than that of the pyridine derivatives.

11 *Pharmacology*

MECHANISMS FOR TERMINATING THE ACTION OF EPINEPHRINE ON THE EMBRYONIC CHICK HEART. Leslie P. McCarty* and Frederick E. Shideman. Dept. of Pharmacology and Cardiopulmonary Research Laboratory. Univ. of Wisconsin, Madison.

Inotropic and chronotropic responses of the embryonic chick heart to epinephrine at different stages of development were studied by means of an apparatus developed in this laboratory. Exposure of hearts from embryos 4 days of age to epinephrine for several minutes produced the characteristic responses which persisted and could be abolished only by repeated washing. These hearts responded less to subsequent exposure to epinephrine. A persistent action of epinephrine was not noted with older, innervated (e.g. 6 day-old) hearts. The 4 day-old heart possessed little or no capacity to metabolize epinephrine; significant activity was observed in the case of the 6 day-old heart and the 15 day-old heart exhibited a still greater capacity to metabolize epinephrine. In the innervated embryonic heart pyrogallol (10^{-4} M) markedly inhibited epinephrine metabolism whereas iproniazid (10^{-4} M) was without a significant effect. These observations suggest that the actions of epinephrine on the innervated embryonic chick heart are terminated primarily via metabolism by means of catechol-O-methyl transferase. Other mechanisms, e.g. tachyphylaxis, may explain the decreased sensitivity to epinephrine in the non-innervated heart following its prior exposure to this drug.

12 *Pharmacology*

THE EFFECT OF EPHEDRINE ON MYOCARDIAL CONTRACTILITY IN DOGS PRETREATED WITH RESERPINE. Joanne I. Moore* and Neil C. Moran. Dept. of Pharmacology, Emory Univ., Atlanta, Georgia.

Burn and Rand (J. Physiol. 144:314, 1958) postulated that the effects of certain sympathomimetic amines are mediated through norepinephrine release. To test this hypothesis, ephedrine augmentation of myocardial contractility was studied in 25 pentobarbital anesthetized, vagotomized, open-chest dogs with strain gauge arches sutured to the right ventricle. Ten animals were pretreated with 0.5 mg/kg I.P. crystalline reserpine prepared with dilute acetic acid, 9 were pretreated with commercial parenteral reserpine (Serpasil), and the remaining dogs served as controls. The initial contractile force was increased in both treated groups. The maximal contractile force developed in response to ephedrine, 0.1 mg/kg, was not altered by reserpine. Change in contractile force produced by ephedrine in the Serpasil treated group was not altered from the change in force produced in the control group. In those dogs pretreated with crystalline reserpine the change in force was significantly decreased. The slow intravenous infusion of norepinephrine at rates of 0.1 to 0.5 ug/kg/min for one hour failed to restore or augment the contractile response to ephedrine. These data are not consistent with the hypothesis that the effects of ephedrine are mediated through norepinephrine release.

13 *Pharmacology*

SOME RELATIONS BETWEEN ELECTRICAL AND MECHANICAL ACTIVITY IN ISOLATED FROG VENTRICLE STRIPS. Emil R. Smith (intr. by D.S. Riggs) Department of Pharmacology, School of Medicine, University of Buffalo, Buffalo, N.Y.

Isometric myograms and monopolar surface electrograms were recorded simultaneously from frog ventricle strips stimulated along their entire length so as to exclude conduction effects. Each strip was stimulated at various rates between 6 and 120/minute. As the rate increased the duration of mechanical latency decreased. The rate of development of tension, the maximum tension, the times to maximum tension and complete relaxation, and the duration of the action potential all increased as the rate of stimulation was moderately increased, but decreased with higher rates of stimulation. However, the rate of development of tension and the time to maximum tension varied independently. The time to maximum tension was directly proportional to the duration of the action potential. It was concluded that the maximum tension developed during systole depends upon two processes: one determines the rate of development of tension and the other determines the time over which it develops. Repolarization always ended after maximum tension had developed, but before relaxation was complete. Therefore, relaxation could not have been initiated by the completion of repolarization. (Supported by USPHS NF-5731-C3)

14 *Pharmacology*

VERATRINE RESPONSE IN THE RAT RIGHT VENTRICLE STRIP. Joseph M. Benforado, Dept. of Pharmacology, University of Buffalo Medical School, Buffalo, N.Y.

In previous experiments on the contracting right ventricle strip of the rat(J.Pharmacol.Exp.Ther., 122:86,1958) a delay in relaxation following veratridine was occasionally noted. This delay resembled the early phase of the veratrine response in skeletal muscle. Using the same preparation previously employed, the effect of veratridine on isometric contractions was studied in an attempt to establish the presence of a veratrine response in cardiac muscle. Frequencies of 1-6/min and drug concentrations of 0.3-10 mcg/ml were used. At low concentrations delayed relaxation was seen; at higher concentrations this gave way to an after-contraction resulting in a two-peaked myogram. The after contraction was usually 10-20% of the initial twitch although in some experiments it approached 40%; its time course often extended to 30 seconds when the rate was 1/min. A complete merging of the after-contraction with the initial twitch was never observed. If the frequency of stimulation was increased, the delayed relaxation decreased and could not be obtained at frequencies of 70-100/min. Increased potassium in the bathing fluid antagonized the effect while decreased potassium enhanced it. Passive length-tension relations were not altered. (Supported by the Heart Assoc. of Erie Co.)

15 *Pharmacology*

MECHANISM OF PENTYLENETETRAZOL-INDUCED CARDIAC ARRHYTHMIAS. R. P. Bircher, T. Kanai* and S. C. Wang. Sandoz Pharmaceuticals, Hanover, N.J. and College of Physicians and Surgeons, Columbia University, New York City.

Pentylenetetrazol was given either i.v. or on the cortex or in the lateral, 3rd or 4th ventricle (via chronically implanted cannulae) of dogs under succinylcholine Cl and artificial respiration. The EEG and the ECG were recorded with a Grass Polygraph. Pentylenetetrazol, 10-30 mg/kg, i.v., produced seizure discharges and various reversible cardiac arrhythmias. 1.5-3 mg/kg pentylenetetrazol applied to the cortex produced similar seizure discharges but no cardiac arrhythmias. 3-5 mg/kg of pentylenetetrazol, injected into the cerebral ventricles and especially the 4th ventricle, caused cardiac arrhythmias with or without seizure discharges. Doses of 1.5-5 mg/kg, i.v., were without effects. Trimethadione, 300-500 mg/kg, i.v.; methyl-phenyl-ethyl hydantoin, 100 mg/kg, i.p.; l-hyoscyamine, 0.1 mg/kg, i.v.; synthetic N-ethyl-nortropine-benzhydryl-ether, 0.2 mg/kg, i.v.; or vagotomy protected against these cardiac arrhythmias. Blockade of the vagal effects did not protect fully in some instances. Additional administration of Hydergine, 0.2 mg/kg, i.v., afforded complete protection. These results suggest that pentylenetetrazol-induced cardiac arrhythmias are of central origin and produced mainly by stimulation of the vagal centers in the floor of the 4th ventricle. (Supported by USPHS Grant B31C.)

066. CARDIOVASCULAR SYSTEM—Cardiac Muscle—Disorders

1 *Physiology*

CARDIAC HYPERTROPHY DUE TO HYPOXIA IN MALE AND FEMALE RATS. David W. Northup and Edward J. Van Liere. W. Va. Univ. Medical Center, Morgantown, W. Va.

It has been shown in our laboratory that the cardiac hypertrophy induced by exercise in the albino rat is significantly greater in the female than in the male. To determine whether the female heart is more sensitive to stress of other kinds, the effect of hypoxia was studied. Nineteen male and 21 female albino rats were exposed to a simulated altitude of 24,000 ft. for eight hours per day for four weeks. Twenty males and 28 females served as controls. At the end of the experimental period, the rats were killed, their hearts weighed, and the heart weight-body weight ratios calculated. There was a significantly greater increase in the ratio in the male rats. It is concluded that sex difference in hypertrophy is dependent on the ability of the organism as a whole to withstand stress, and not to a difference in the sensitivity of the heart.

XUM

2 *Physiology*

THE QUANTITATIVE ESTIMATION OF MYOCARDIAL INFARCT SIZE BY ELECTROLYTE SHIFTS AND BY SGO-TRANSAMINASE. R. A. Russell,* A. J. Bocage* and A. S. Harris. L.S.U. Sch. Med., New Orleans, Louisiana.

For certain studies of experimental myocardial infarction a precise determination of infarct size is useful. Techniques have been developed whereby the potassium loss from, and the sodium influx into, the damaged muscle may be used to estimate the severity of myocardial injury. The left anterior descending coronary artery of the dog was occluded by tightening a loop carried within a fine catheter, a week after its surgical placement. This technique makes occlusion possible without anesthesia, opened chest and artificial respiration, or simultaneous skeletal muscle damage. SGO-transaminase determinations were also done, at 4 hour intervals. It was found that SGO-T concentrations rise rapidly to a peak at about 14 hours after occlusion, then fall at a rate such that in 24 hours the concentration is little more than half the maximum. Hence to gain meaningful information about infarct size from transaminase data the time after occlusion must be accurately known, and/or serial blood analyses must be performed. The two methods, electrolyte shifts after 24 hours and SGO-T maxima, show fair correlations over a wide range of infarct sizes. (Aided by grant No. H-1109, U.S.P.H.S.)

3 *Pharmacology*

ION FLUX IN CARDIAC ARRHYTHMIAS. R.B.Arora.(Intr. by Arthur Grollman,) Department of Pharmacology, All India Institute of Medical Sciences, New Delhi, India.

Experimental cardiac arrhythmias were produced in 93 mongrel dogs of either sex (12-18 Kg.wt.) under pentobarbitone (30 mg /kg. i.v.) anesthesia. Samples of blood, arterial (from ascending aorta) and venous (from coronary sinus) were analysed for sodium, potassium, magnesium and calcium. The rise in serum concentration of sodium, potassium and calcium in aconitine and acetylcholine induced auricular fibrillation and in injury stimulation auricular flutter was nonspecific in nature (t test, range of $P<0,9-0.1$). However, a specific efflux of magnesium from the heart was obtained in aconitine and acetylcholine induced auricular fibrillation ($P<0.001$ and 0.02 respectively), but not in auricular flutter($P<0.8$). This indicated that magnesium efflux from the heart was a specific phenomenon and was not due to rapid heart rate. Similar findings were obtained when all the three arrhythmias were produced in the same animal one after the other. In ouabain evoked ventricular tachycardia, a specific efflux of potassium and magnesium ($P<0.02$ for both) was observed. It was concluded that experimental cardiac arrhythmias produced by different techniques may have different mechanisms.

4 *Pharmacology*

TRANSMEMBRANE FLUXES IN VENTRICULAR FIBRILLATION. A.H. Briggs*and W.C. Holland. Dept. of Pharmacology, Univ. of Miss. Med. School, Jackson, Mississippi.

Fibrillation was induced by stimulating electrically in low extracellular potassium concentration $(K)_o$. K efflux increased from 10.6 pmoles $cm^{-2}sec^{-1}$ in normally beating controls to 14.0 pmoles $cm^{-2}sec^{-1}$ during fibrillation. K influx decreases from 2.5 pmoles $cm^{-2}sec^{-1}$ in controls to 1.2 pmoles $cm^{-2}sec^{-1}$ during fibrillation. The rates of entry of Na increased 5 times, Cl increased 2.2 times and Ca increased 4.6 times during fibrillation. Examination of efflux curves of these three ions indicated that the increases were due to an increased influx. Lowering the $(K)_o$ results in a much greater loss in ventricular $(K)_i$ than in atrial tissue. ACh plus electrical stimulation (1200/min) increases the rate of atrial $(K)_i$ loss to levels comparable to the rate in ventricular muscle in low $(K)_o$. A further 50% increase in the net loss of K results in fibrillation in both tissues. Atrial and ventricular fibrillation begin when the rate of loss of K and gain of Na exceeds a critical one. Ventricular tissue reaches this critical rate rapidly when the $(K)_o$ is lowered and is thus extremely susceptable to fibrillation.

5 *Pharmacology*

ANION INTERACTION IN ISOLATED ATRIA. W.C. Holland and A.A. Sekul*. Dept. of Pharmacology, Univ. of Miss. Medical School, Jackson, Mississippi.

Substitution of various anions for Cl^- reduced the incidence of acetylcholine (ACh) induced fibrillation, increased the refractory period and voltage threshold of isolated rabbit atria. The order of effectiveness being $SCN^- > I^- > Br^- > NO_3^-$. The changes induced are reversed within a time consistent with extracellular equilibration. Anion replacement leads to a slight increase in K efflux, a moderate reduction in Na influx and profound fall in Cl influx. No effects were noted on Ca exchange. Replacement of 3/4 the Cl^- by NO_3^- or 1/9 by SCN^- reduced Cl influx 60% and Na influx only 20%. There is evidently preferential concentration of NO_3^- and still more SCN^- in a region where Cl^- influx is impeded; probably the cell membrane. It is concluded that the effects of foreign anions on the electrical properties of atria are a result of their ability to impede Cl^- influx and not to an action on Na exchange. Similarities and differences between anion replacement and quinidine will be discussed.

067. CARDIOVASCULAR SYSTEM–Cardiac Muscle–Electrocardiography

1 *Physiology*

EFFECT OF PSYCHOLOGICAL STRESS ON HEART RHYTHM. R.B. Pearson, Janesta Janzen, and Richard Sturdevant (intr. by Vernon C. Bohr) College of Medical Evangelists, Loma Linda, Calif.

Changes in average heart rate and rhythm accompanying emotional disturbance have been described. To further investigate rhythmic behavior a method was devised which affords a concise, high resolution display of the intervals in a train of events. Such displays were developed on human subjects during attempts to induce psychological stress by solely verbal means. These showed frequent episodes of stabilization of the heart rhythm which did not occur during the control periods. Certain interesting features characterize this phenomenon: a) correlation of onset and recovery in time with the stress situation, b) abruptness of onset and recovery, c) reduction of total "jitter" to as low as 1/20 its initial value, d) sinus arrhythmia commonly abolished, e) positive correlation with PGR f) variable increase in average rate. Epinephrine of sufficient dosage to produce the same order of rate increase failed to produce comparable stabilization. Immediately following exercise a degree of stabilization similar to psychological stress was produced, however, some sinus arrhythmia persisted. A tentative hypothesis is that this reaction derives from a psychosomatic reflex of neurogenic mediation which exhibits a relatively discrete threshold which is exceeded during peaks of psychological stress. *Deviation from absolute rhythm

2 *Physiology*

DIFFERENCES IN THE EFFECT OF ELECTROLYTES ON THE ATRIAL AND VENTRICULAR ACTION POTENTIALS OF ISOLATED, PERFUSED RABBIT HEARTS. E.Lepeschkin, B.Surawicz*and H.C.Herrlich*. Div.ofExp. Med., Univ. of Vermont College of Med., Burlington, Vt.

Electrocardiograms and monophasic action potentials registered with suction electrodes from the atrium and ventricle were recorded continuously after a rapid change in the composition of the perfusing solution. The atrial action potential is characterized by a short phase 2 ("plateau") and a long slow terminal descending phase 4, while in the ventricle phase 2 is long and phase 4 short. Low external potassium causes phase 3 to appear earlier and become less steep, accentuating phase 4 in the atrium less than in the ventricle. High external potassium causes phase 3 to proceed more rapidly, decreasing phase 4 and the amplitude and slope of ascent; this effect is greater in the atrium. If magnesium is low, low external calcium in the ventricle prolongs phase 2, causing a marked increase in total duration, but in the atrium it has at first little effect on phase 2 but abolishes phase 4, causing marked decrease in duration; later phase 2 is prolonged and the total duration increased as in the ventricle. These differences are therefore more quantitative than qualitative; they may be related to a higher calcium content and a greater sensitivity to acetyl choline in atrial muscle.

3 **Physiology**

ORS AND T AXES IN BEEF CATTLE. Hugo Krueger, Wm. C. Van Arsdel III*, and Ralph Bogart*. Dairy and Animal Husbandry, Oregon State College, Corvallis, Oregon.

The ORS vector for Calf F-25 may be expressed as $-4.0\underline{i} + 8.1\underline{j} + 2.4\underline{k}$. The vector may also be expressed as having an azimuth angle of 60° from the left arm toward the head in the L (leg) plane, a 60° angle of elevation, and a magnitude of 0.93 millivolts. The vector points dorsad, cephalad, and to the left. The T vector may be expressed as $-1.2\underline{i} - 2.8\underline{j} - 1.5\underline{k}$, with an azimuth angle of 144° (rotating from the left arm toward the head and then toward the right arm) an angle of depression of 54°, and a magnitude of 0.34 millivolts. The T vector points ventrad, cephalad, and to the right. The QRS and T vectors have an angle of 134° between them. The P vector may be expressed as $1.4\underline{i} - 0.8\underline{j} + 0.16\underline{k}$, with an azimuth angle of 79° from the left arm toward the tail, an angle of depression of 60° and a magnitude of 0.16 millivolts. The vector points ventrad, caudad and slightly to the left.

The average QRS angle in the S plane shows little change between 500 and 800 pounds body weight. The QRS angle was not significantly different in males and females. However, Prince calves had smaller QRS angles than did the Davids, or the Lionhearts. (Physiologist 2, 74; Oregon Agricultural Experiment Station Bulletin 51).

4 **Physiology**

DEPOLARIZATION AND REPOLARIZATION IN THE HYPOTHERMIC DOG HEART J. C. Torres,* E.T. Angelakos, and A.H. Hegnauer, Department of Physiology, Boston Univ. School of Med., Boston, Mass.

Depolarization and repolarization potentials were studied using the QRS and T deflections of leads I and AVF during continuous cooling. Data analysis revealed that hypothermia effected a moderate increase in the area of the depolarization potential along with a decrease in the rate of conduction as evidenced by progressive increase in duration of the QRS. This depressant effect of cold on conduction velocity was uniform, since electronic differentiation of the QRS complex revealed no abrupt changes in rate of formation down to 21°C. Below this temperature there was evidence of non-uniform conduction. The frontal projection of the excitation potential remained quite stable demonstrating that the direction of excitation remained constant. Repolarization potentials increased considerably but were inconsistent in orientation down to 23°C. Below this temperature the course of repolarization tended to oppose that of depolarization. Increasing heart rate via an auricular electrode produced no significant alterations in any measured parameter. These results suggest that while the excitation process is uniformly and consistently affected by moderate cold, the recovery process is extremely thermolabile and demonstrates great variability. (Supported by American Heart Association.)

5 **Physiology**

MYOCARDIAL ECGs IN LOCALIZED ISCHEMIA. J.J. Sayen, A.H.Katcher*, G. Peirce,*W.F. Sheldon,*and E.H.Christman.*Univ.of Pa.,Phila.

In open-chest dogs myocardial ECGs were recorded from 0.2mm Pt electrodes (AC amplifier) at 3-10mms depths, or from Ag-AgCl electrodes (DC amplifier). Coronary branch occlusion caused RS-T segment elevation and decreasing S-wave amplitude concurrent with local O_2 fall and disturbed contractility. DC records from the centers of the ischemic areas showed net "RS-T shifts" were baseline depression, with RS-T elevation absent or late and slight. Negative RS-T displacement attributable to local ischemic changes was seen nowhere. 50% procaine applied to limited areas of exposed ventricular surface elevated the RS-T segment only in the most superficial layers of underlying muscle. Posterior wall application caused similar changes locally and in Lead II, but smaller reciprocal changes in all direct leads remote from the area of primary effect. In contrast, topical 5% KCl caused locally negative and reciprocally positive baseline shifts. Posterior wall insulation from the pericardial cradle abolished reciprocal and Lead II changes. If reciprocal effects are avoided (by limiting ischemia to the exposed anterior heart surface) or detected (with the aid of DC amplifier records and posterior wall insulation) myocardial ECGs are prompt, sensitive indices of local muscle change, especially valuable for study of early or labile ischemic situations. (Supported by USP grants H-398 and HF-7812.)

6 **Physiology**

RELATIONSHIP OF BLOOD PRESSURE AND ECG AMPLITUDES AND SPATIAL VECTORS IN HEALTHY SUBJECTS. Pentti Rautaharju and Henry W. Blackburn (intr. by H. L. Taylor.) Lab. Phys. Hygiene, U. of Minn., Minneapolis.

Relationship of blood pressure, ECG amplitudes and mean spatial vectors was analyzed in 468 laborers, ages 20 - 60, putatively healthy. Values by age were compared in three blood pressure groups: A, under 140/90 mm. Hg (n=215); B, 140/90 to 160/95 (n=146); C, 160/95 or over (n=107). No significant differences in R or T amplitudes were found, but correlation of R and T revealed a significant difference in intercept between lowest and highest pressure groups. QRS spatial angle of elevation revealed a more "horizontal electrical position" in groups B and C. The significant positive correlations of QRS elevation angle, and the angle separating QRS and T vectors, with blood pressure was unchanged after eliminating effects of age and relative weight. The age trend of QRS elevation was not apparent after elimination of blood pressure effect. These electrical tendencies, sensitive indicators of left heart hypertrophy and ischemia in hypertensive patients, are also found in asymptomatic workers with moderate blood pressure elevation.

7 **Physiology**

INTRAVENTRICULAR CONDUCTION DISTURBANCES IN ACUTE MYOCARDIAL INFARCTION. Maria Isabel Rodriguez. School of Medicine. University of El Salvador.

Studies had been made within 24 hours after ligature of the left coronary artery in 24 dogs. The electrical phenomena were continuously registered during a minimum of 8 hours up to a maximun of 24. In each case were simultaneously registered one standard lead II and unipolar leads in several points of the epicardial surface; moreover, in ten cases, the conduction was also studied in each millimeter of the heart wall using fine electrodes. Typical curves were observed in 16 cases; in 8, atypical curves were obtained during the period of observation. These curves are caractherized by a) enlargement and slurring of QRS complex, b) no displacement of ST segment, c) similarity with curves of incomplete left bundle branch block, d) these changes occur only in the infarction area; records from inmediately contiguous areas show normal QRS complexes. The curves described herein correspond to slow propagation of the impulse in injury tissue as shown by Histological sections. The changes might be classified as focal block and could appear in myocardial infarction instead of classical monophasic waves.

068. CARDIOVASCULAR SYSTEM–Cardiac Muscle–Excitability

1 **Physiology**

ACCOMMODATION IN THE MAMMALIAN HEART. C. McC. Brooks, J.L. Gilbert (by invitation), G. Lange (by invitation) and E.E. Suckling. Downstate Medical Center, State University of New York.

By determining change in threshold to one msec. rectangular test stimuli produced by long duration (50-70 msec.) subthreshold conditioning rectangular pulses applied to the heart of anesthetized dogs (chloralose-urethane; nembutal) during late diastole it was found that ventricular muscle demonstrates the power of accommodation. Maximum facilitation or lowering of threshold occurred within 15 msec. after application of a 70 msec. conditioning stimulus of 80% threshold value. By the end of 20-25 msec. this facilitating action began to diminish and the threshold requirement for testing stimuli began to rise. This process of accommodation was observed to occur in the exposed heart as well as in the unexposed heart stimulated by means of implanted electrodes. The conditioning current pulses were monitored and found to maintain their rectangular form. Accommodation occurred when current was applied either through small silver electrodes or through non-polarizable wick electrode. There was great variation in the extent and speed of accommodation observed. Factors of importance to this reaction are being studied and it can be said that the blood CO_2 content directly or indirectly affects accommodation. (Supported by a grant from the Life Insurance Medical Research Fund.)

XUM

2 **Physiology**

NON-UNIFORM TWO-DIMENSIONAL ELECTROTONIC SPREAD IN RAT ATRIUM. W. E. Crill* and J. W. Woodbury. Dept. of Physiology & Biophysics, Univ. of Washington School of Med., Seattle, Wash.

Two-dimensional spread of current (I) from a fixed intracellular stimulating electrode was mapped in excised portions of rat auricular appendages. Spread was estimated by measuring the changes in transmembrane potential (V) with another microelectrode inserted at various distances from and angles to the I electrode. The time course of the potential changes was similar to those found in long thin fibers. The radial spatial decrement was approximately exponential, but the space constant (L) depended on direction. L was about 150 μ in the direction of the roughly parallel fiber bundles and about 100 μ at right angles. Equivalue V/I contours were irregularly shaped. Current spread farthest in the fiber direction, but the direction of least spread was not predictable. The ratio of the radii of greatest (r_1) to least (r_2) spread at V/I = 10,000 ohms was about 2.5; r_1 = 200 μ. This two-dimensional pattern is expected from the ultrastructure of cardiac tissue. In such a structure extracellular resistance must be much higher than intracellular and the space constant would be about 100 μ. Potential changes in cells other than the one impaled by the I electrode could be induced by large potential drops in the extracellular fluid. Since the cell's long dimension is of the order of L, the irregularities in the V/I contours might indicate cell boundaries.

3 **Physiology**

TRANSMEMBRANE ACTION POTENTIALS OF SPECIALIZED FIBERS IN RAT HEART. Zia J. Penefsky,* and Brian F. Hoffman. Dept. of Physiology, State Univ. of New York, Downstate Med. Center, Bklyn, N.Y.

Studies were made of bundles of fibers running free in the left ventricular cavity. Anatomically, these fibers are continuations of the His bundle, forming a network in the ventricular cavity and ending in the papillary muscles or the ventricular wall. Histologically, these fibers are pale and large, with few myofibrils. They selectively stain with methylene blue. Transmembrane action potentials recorded from different parts of the network show 3 distinct shapes. In an area close to the His bundle the resting potential is 60-70 mv. and the reversal 40 mv. Repolarization to 50% of the resting potential occurs in 1-2 msec. Subsequently, a plateau of 30 msec. duration precedes completion of repolarization. In an intermediate area the resting potential and reversal are of similar magnitude. However, the initial phase of repolarization is less rapid and the plateau appears at a lower level of membrane potential. Close to the junction with ventricular muscle the resting potential is 70-80 mv. and the reversal 25-30 mv; no clear plateau is recorded from these fibers. Nor-epinephrine causes pacemaker activity in the fibers closest to the His bundle and in addition causes changes in the action potential not directly attributable to slow depolarization.

4 **Physiology**

THE EFFECT OF HISTAMINE-RELEASE AGENTS ON VENTRICULAR TACHYCARDIA FOLLOWING MYOCARDIAL INFARCTION. Jessie E. Hano (intr. by Richard Ashman.) L.S.U. Sch. Med., New Orleans, La.

Potassium and histamine have been considered possible excitants of ventricular arrhythmias following myocardial infarction. Infarction was produced in dogs by ligation of the anterior descending coronary artery by the Harris two stage technique. A catheter was inserted distal to the ligation for later injections into the ischemic area. Compound 48/80 and n-octylamine were injected into non-occluded arteries and into the ischemic bed of 1 and 24 hr. infarcts. Serial EKG's were recorded, and animals were sacrificed 30 min. after injection. Na and K were determined on digests of normal and infarcted muscle of each heart. In all preparations 48/80 produced no change in ventricular rhythm or myocardial electrolytes. Octylamine, which releases both K and histamine, produced ventricular ectopic rhythms and fibrillation with decreased K concentration in non-occluded and 1 hr. infarcted dogs, but failed to affect the ventricular rhythm 24 hrs. after infarction, although it reduced the K to serum levels. It appears that the release of endogenous histamine does not play a major role in the genesis of arrhythmias following infarction. Potassium apparently is intimately related to ectopic rhythms immediately following coronary occlusion, but probably does not function prominently in the ventricular tachycardia seen 24 hrs. later. (Supported in part by grant No. H-1109 U.S.P.H.S.)

5 **Physiology**

VENTRICULAR THRESHOLDS FOR NON-RECTANGULAR ELECTRICAL STIMULI. Guy Knickerbocker and William R. Milnor (intr. by Samuel A. Talbot) Johns Hopkins Univ., Baltimore, Maryland.

"Square waves" are commonly used to study myocardial excitability, limiting stimulus variables to current strength and duration. Stimulus "rise-time", or rate of increase of current to maximum, is a third important variable, which is of theoretic interest because of its probable relation to cell membrane capacitance, and of practical interest because most artificial pacemakers in clinical use do not employ square waves. Ventricular thresholds for trapezoidal waves were measured, varying the stimulus rise-time, using contiguous bipolar stimulus electrodes on the epicardium of open-chest dogs. As expected, the longer the rise-time the higher the threshold. Strength-duration curves for trapezoidal waves had the same contour as those for square waves. Although it has been reported that very slowly rising currents fail to stimulate nerve, regardless of the maximum current reached, this phenomenon could not be demonstrated in our experiments. The slowest rate of rise tested (0.01 milliamperes/millisecond) evoked a response, although the peak current required was 3 times that needed for square waves of equal total energy.

6 **Physiology**

INTRACELLULAR ACTION POTENTIALS AND CONTRACTIONS OF HUMAN ATRIAL STRIPS IN VITRO. William Sleator, Jr., T. de Gubareff* and V. Krespi*. Physiology Dept., Washington University, St. Louis, Mo.

Pieces of atrial muscle about 1 mm. thick, removed from patients undergoing heart surgery, were suspended in oxygenated Krebs bicarb. Ringer's and stimulated electrically. Contractions and intracellular action potentials were simultaneously recorded. All preparations (45 patients) showed a type of behavior which has not been seen in heart muscle of any other species. When all conditions in the bath, including stimulation frequency, are kept constant, these bundles do not approach a steady state, but alternate periodically between 2 phases with contractions which may differ as much as 8 fold. High phase contractions are multiple responses associated with A. P.'s having multiple spikes rising from a long plateau near threshold voltage; low phase A. P.'s have 1 spike. At each moment, all cells showed the same type of A. P. The phenomenon can occur in a piece of any size, and at any temperature from 15 to 37°C. The strong contractions probably constitute an example of "post tetanic potentiation". (U. S. P. H. S. grant B-937.)

7 **Physiology**

ENHANCEMENT OF POTASSIUM-INDUCED A-V BLOCK BY PRIOR INTOXICATION WITH DIGITOXIN. Charles Fisch, B. L. Martz and Fred H. Priebe (intr. by Kenneth G. Kohlstaedt.) Lilly Lab. for Clinical Research, Marion Co. General Hospital, and Dept. of Med., Indiana Univ. School of Med., Indianapolis, Ind.

Eight mongrel dogs were given a buffered solution of K phosphate (145 mEq./L.) intravenously at a rate of 1.0 mEq./min. until 2° A-V block occurred. These animals were then given 0.5 mg./kg. (not to exceed 5.0 mg.) of digitoxin I.M. 48 hours after the digitoxin, the K phosphate was again infused until 2° A-V block occurred. Prior to administration of digitoxin - 12.7-26.3 (av. 17) mEq. of K was given prior to appearance of 2° A-V block. After digitoxin - 2° A-V block occurred after infusion of 4.8-9.7 mEq. of K (av. 9). This amounted to a diminution of K necessary to produce block of 34.6-77.2 (av. 59.5) percent. The plasma level of K at time of block prior to digitoxin averaged 8.1 (7.3-9.0); after digitoxin, averaged 6.7 (6.3-7.1) mEq./L. The results indicate that A-V conduction system is more sensitive to the depressing effect of K after intoxication with digitoxin.

069. CARDIOVASCULAR SYSTEM—Cardiac Muscle—Metabolism

8 **Pharmacology**

CHRONOTROPIC RESPONSES TO QUINIDINE IN DOGS WITH COMPLETE HEART BLOCK. Richard Stadter*, Michael Olichney*, Jay Roberts and Walter Modell. Cornell Univ. Medical College, New York.

Doses of 5 to 20 mg/kg of quinidine IV increased both auricular and ventricular rates. After 5 mg/kg, the auricular rate rose from 150 to 181 beats/min, the ventricular rate from 51 to 57. While larger doses of quinidine did not increase auricular rate further, ventricular rate was accelerated to about 70 beats/min. These effects developed promptly and disappeared within 15 to 20 minutes. It was demonstrated by pretreatment with Eumydrin that vagolysis played a role only in auricular acceleration after quinidine. Treatment with hexamethonium (2 mg/kg, IV) and reserpine (0.1 mg/kg, IV) slowed auricular rates to about 130 and ventricular rates to about 20 beats/min. Subsequent administration of quinidine further slowed auricular and ventricular rates; in some cases producing periods of auricular or ventricular asystole. The observation that reserpine and hexamethonium reduce auricular and ventricular rates while asystole is produced only when quinidine is also administered suggests that in addition to catechol amine activity, there may be another factor which contributes to rhythmicity of cardiac tissue. The latter is depressed by quinidine, but this effect may be masked by the action of quinidine which increases sympathetic activity. (Supported by USPHS grant H 1165).

9 **Pharmacology**

EFFECTS OF CHANGING EXTERNAL NA AND K CONCENTRATIONS, AND OF QUINIDINE, ON REFRACTORY PERIOD OF RABBIT ATRIUM. David G. Teiger, (intr. by J. Tepperman) Dept. of Pharmacology, SUNY, Upstate Medical Center, Syracuse, N. Y.

Isolated left atria of rabbits were driven at a rate of 1 per second (S_1). Test stimulii (S_2) of increasing strength were applied at decreasing intervals following an S_1. The absolute refractory was recorded as the shortest effective S_1-S_2 interval. The duration of the refractory period is related inversely to the concentration of external Na and directly to the concentration of external K. Quinidine was studied because it has been suggested to act on ionic mechanisms. Quinidine increases the duration of the refractory period. The increased refractory period due to increased external K can be reversed by increased external Na; but the increased refractory period due to quinidine cannot be reversed by Na. To determine how much of the effect of increasing Na concentration is due to hypertonicity alone, sucrose and choline chloride were used. Sucrose hypertonicity decreases the duration of the refractory period to about the same extent as Na (except at the highest tonicities); while hypertonicity due to choline chloride does not decrease the refractory period. Neither substance reverses the effect of increased K concentration on the refractory period.

10 **Pharmacology**

CARDIAC ARRHYTHMIAS PROVOKED BY FOCAL APPLICATION OF DELPHININE. David Scherf, Serge Blumenfeld, Dogan Taner and Muhtar Yildiz (intr. by David Lehr.) New York Medical College, New York.

Delphinine, derived from the seeds of delphinium staphisagria, was applied focally to the atrium of the exposed heart of the dog. In all 23 experiments atrial flutter appeared within 3 minutes and persisted for 30 to 60 minutes. Stimulation of the vagus nerve increased the flutter rate. In five experiments prior to the appearance of flutter, a paroxysmal atrial tachycardia was observed which suddenly stopped when the vagus was stimulated and started suddenly again after the end of the stimulation. Mechanical pressure exerted on the focus on which the delphinine was applied temporarily caused fibrillation. Application of a ligature across any possible pathway around the venae cavae did not influence the existing flutter or fibrillation. Cooling the focus of application or clamping it off from the rest of the atrium immediately stopped the flutter. There was no evidence of a circus movement. (Supported by a grant from the National Heart Institute, United States Public Health Service.)

1 **Physiology**

LABELED PHOSPHATE DISTRIBUTION IN DOG MYOCARDIUM. N. J. Zeig*, K. K. Tsuboi* and N. M. Buckley, Albert Einstein Coll. of Med. and Cornell Univ. Med. Coll., N.Y., N.Y.

The comparative metabolism of phosphate compounds in working and nonworking myocardium was examined with the aid of P^{32} labeled phosphate. Six isolated left ventricle preparations were used in which the nonworking right ventricles served as controls. Isotope perfusion was carried out for fixed periods of time, and excess isotope of extracellular origin subsequently removed. Heart muscle was fractionated and the phosphorylated labeled components ultimately resolved by paper chromatography for specific activity determinations. The nucleotide and nonnucleotide phosphate composition of working and nonworking myocardium was extensively examined. No differences were found with respect to nucleotide and nonnucleotide soluble components. Phosphate P^{32} incorporation into these compounds as well as phospholipids and RNA was investigated in relation to cardiac work. Incorporation of the label into the rapidly exchanged high energy phosphate groups was found to be restricted by a prior rate-limiting entry of isotope into the cells. (Supported by grants from U.S. Public Health Service, Commonwealth Fund and Life Insurance Med. Res. Fund.)

2 **Physiology**

THE OXYGEN UPTAKE OF THE PURKINJE FIBERS OF THE DOG HEART. Kalman Greenspan* and Paul F. Cranefield. Dept. of Physiology, State Univ. of New York, Downstate Medical Center, Brooklyn, N.Y.

The rate of oxygen uptake of quiescent Purkinje fibers of the dog's heart was determined using a flow respirometer and oxygen polarography. At ambient oxygen concentrations of 60% or higher the rate of uptake was 0.739 mm^3/mg. wet weight/hour at 35°C. The temperature coefficient over the range 25°C.-35°C. was 2.3. The uptake was independent of the ambient oxygen concentration at oxygen concentrations equal to or greater than 60% of an atmosphere. Thus at 30°C. the QO_2 at an ambient oxygen concentration of 95% was 0.543 and 0.521 in 60% oxygen. In lower oxygen concentrations the rate of uptake was found to be depressed. In 40% oxygen the rate of uptake was 0.413 mm^3/mg. wet weight/hour while in 20% oxygen it was 0.250 mm^3/mg. wet weight/hour. This reduction of uptake in the lower oxygen tensions is probably the result of diffusion limitation. Our results do not exclude the possibility that the rate of uptake is depressed by a low oxygen tension. (Supported by USPH grants H-3916 and H-1250).

3 **Physiology**

THE PRODUCTION OF AMMONIA IN PERFUSED HYPOTHERMIC RABBIT HEARTS. H. Feinberg and Sister Mary Alma*. Cardio. Dept., Michael Reese Hosp. and Med. Center, Chicago, Ill. and Notre Dame College of Maryland, Baltimore, Md.

The net production of ammonia by perfused cardiac muscle shown by Embden in the frog is confirmed in the rabbit. Oxygenated Krebs solution was perfused through the coronary system of the hypothermic, working rabbit heart. Left ventricular pressure, heart rate, perfusate flow and ammonia production were measured. Observations were made before and after epinephrine and adenosine addition to the perfusate. A rough correlation appeared between the parameters of cardiac effort and ammonia production. Epinephrine increased perfusate flow, cardiac effort and ammonia production; the increase in ammonia production being disproportionate relative to the increased cardiac effort. Adenosine, while increasing perfusate flow, had no consistent effect on cardiac effort nor on ammonia production.

XUM

4 *Physiology*

MYOCARDIAL NUCLEOTIDES AND CREATINE PHOSPHATE IN ANOXIC CARDIAC ARREST. Ellis S. Benson*, Gerald T. Evans, Ben E. Hallaway* and Clifford Phibbs*. Depts. of Laboratory Medicine and Surgery Univ. of Minnesota, Minneapolis.

Cardiac arrest was induced in Langendorff preparations in dogs by perfusion with PVP-Ringer's solution. After 45 min., 26°C., samples of right ventricle were frozen in liquid N_2 and analyzed for CrP by method of Furchgott (J.B.C. 223:377, 1956) and nucleotides by method of Hurlbert et al (J.B.C.209:23,'54)

On recovery, by perfusion with oxygenated blood, heart beat was feeble but in most instances regular (rate 20-40). Samples were taken after 30 min. All results are for right ventricle and in μM/gram heart (see table), number of dogs in each group are in parentheses. With decrease of total nucleotide from 4.8 to 3.9 in anoxia and 2.6 in recovery, inosine and hypoxanthine appeared in heart muscle and effluent.

		CrP	ATP	ADP	AMP	Inorg. P
Control working	(7)	3.0± .3	4.3±.6	1.3±.2	0.5±.2	10.5±1.8
" non-work., perfused	(4)	9.7	3.5	1.0	0.2	7.1
Anoxic;arrested	(11)	1.7± .6	2.5±.6	1.0±.3	0.4±.2	13.7±4.1
Recov.,reg. beat	(12)	7.0±2.1	1.8±.6	0.6±.2	0.2±.2	8.0±2.2
" ., fibrill.	(4)	3.3±1.8	1.2±.6	0.6±.3	0.3±.1	13.8±2.4

Aided by USPHS grant 1584.

5 *Biochemistry*

ROLE OF HYPOTHERMIA IN THE PROTECTION OF MYOCARDIAL METABOLISM DURING CARDIO-PULMONARY BY-PASS AND ELECTIVE CARDIAC ARREST. R. G. Ellison,* S. A. Singal, W. H. Moretz,* E. L. Brackney,* W. F. Butler,*W. C. Maloy,*and D. P. Hall.* Medical College of Georgia, Augusta, Georgia.

Myocardial metabolism was studied in dogs during elective cardiac arrest for 30 min. while on C-P by-pass. Arrest was produced by the following methods: (1) Hypoxia by simple occlusion of ascending aorta, (2) 2½% potassium citrate in 10% glucose, (3) 2½% potassium citrate with adenosine triphosphate and (4) hypoxia with hypothermia. Myocardial biopsies were obtained before arrest, after 30 min. arrest with no coronary flow and at varying periods of time after restoration of coronary flow and cardiac resuscitation. Biopsies were analyzed for glycogen, lactic acid, phosphocreatine and A.T.P. Lactic acid levels increased greatly in all groups. Glycogen decreased in all groups except when hypothermia was used. Decrease in phosphocreatine values occurred in all cases, but to a much less degree in the hypothermia dogs. A general decrease in A.T.P. values occurred in all cases. The value of the addition of 10% glucose or A.T.P. to the arresting solution was not determined. These results suggest that hypothermia provides some protection for myocardial metabolism during elective hypoxic arrest. (Supported by Ga. Heart Assn., PHS research grant H-2894 and Prof.Res.Fund,Med.College of Ga.)

6 *Pharmacology*

METABOLIC STUDIES OF EXPERIMENTALLY INDUCED HEART FAILURE IN THE GUINEA PIG. Arnold Schwartz* and Kwang S. Lee. Dep't. of Pharmacology, State Univ. of N.Y., Downstate Med. Ctr., Bklyn. 3, N.Y.

"Chronic" congestive heart failure was experimentally produced in guinea pigs in a 5 to 7 day period by coarctation of the proximal portion of the ascending aorta. Mitochondria were obtained from the heart, liver, and kidney of the experimental and control animals. P:O ratios, utilizing glutamate or succinate as a substrate were measured by the Warburg technique. ATPase activity of the mitochondria was measured by the method of Racker (Ann. NY Acad. Sci. 72:429, 1959). The P:O ratio of mitochondria obtained from the experimental animal was found to be lower than that of the control animal. However, there was no difference in the ATPase activities. On the other hand in "acute" heart failure produced by more severe coarctation, the P:O ratios of these heart mitochondria were essentially similar to that found for the mitochondria of the control series. These data would suggest that the biochemical state of the mitochondria differ in the "chronically" and "acutely" failed heart. (Supported by USPHS grant H-2875).

7 *Pharmacology*

ACETYLCHOLINE AND CARDIAC GLYCOGENOLYSIS INDUCED BY THEOPHYLLINE OR ANOXIA. Norma H. Vincent* and Sydney Ellis. Dept. of Pharmacology, Woman's Medical College of Penna., Philadelphia.

In a previous communication (The Pharmacologist 1: 62, 1959) it was reported that acetylcholine antagonizes the glycogenolytic effect of epinephrine upon the isolated guinea pig heart. In order to circumscribe the metabolic site of action of acetylcholine, the action of acetylcholine on glycogenolysis produced by other means was investigated. Langendorff preparations of male guinea pig hearts were perfused with Krebs-bicarbonate solution at 37.5°C. Perfusion of the heart with theophylline 0.75 mg/ml for one hour caused an increase in rate and an initial increase in amplitude followed by depression with marked glycogenolysis. Infusion of acetylcholine, 1.8 mcg/min, with theophylline caused a decrease in rate and amplitude, but the glycogen levels were equal to those of control hearts. When hearts were perfused for one hour in 95% N_2-5% CO_2 or in N_2-CO_2 and infused with acetylcholine, 18 mcg/min, they showed a severe decrease in rate and amplitude and low glycogen content. Since epinephrine and theophylline activate phosphorylase but anoxia does not, acetylcholine may interfere with glycogenolysis in that complex system which controls the level of cardiac phosphorylase activity. (Supported in part by U.S.P.H.S. Grant B-1657.)

8 *Pharmacology*

THE POLAROGRAPHY OF ADENOSINETRIPHOSPHATE. Charles D. Proctor. Stritch School of Med. and Grad. School, Loyola Univ., Chicago, Ill.

Polarographic characteristics of adenosinetriphosphate (ATP) have been investigated in 0.1 N perchloric acid supporting electrolyte. A Sargent Model XXI recording polarograph was used to record polarograms. Determinations were made at a cell temperature of 25° C. ± 0.1° and with a dropping mercury electrode (D.M.E.) having a capillary constant ($m^2/3t1/6$) of 2.415 mgm2/3sec1/6. Half-wave potentials for ATP range from -1.1V to -1.2V dependent on ATP concentration and solution pH. The reduction of ATP at the D.M.E. appears to be associated with the adenine portion of the molecule, as fifteen 5'ribonucleotides not containing adenine (inclusive of cytidine, uridine, guanosine, inosine, and cytosine derivatives) tested to date have been found to be inactive at the D.M.E. Waves yielded by ATP appear to be characteristic for amino substitution on the 6-position (ie., no waves are given by either hypoxanthine or cytosine). Diffusion current for the ATP waves was found to be proportional to concentration of ATP over the range tested (0.1mM-0.5mM). (Supported by grant from the Chicago Heart Association.)

9 *Pharmacology*

ACTION OF DIGOXIN AND INSULIN ON THE GLUCOSE TRANSPORT OF THE MYOCARDIUM. **Gerald A. Kien,* A. W. Gomoll* and T. R. Sherrod. University of Illinois, College of Med., Dept. of Pharmacology, Chicago, Illinois.**

Previous investigations in this laboratory have shown that administration of therapeutic doses of digoxin to the intact anesthetized dog results in an increased rate of myocardial metabolism, presumably as a result of an augmentation of carbohydrate metabolism (Circ. Res., In Press). From these studies it was concluded that this cardiac glycoside might possibly influence carbohydrate metabolism by influencing one or more of the rate limiting steps of the Embden-Meyerhof pathway. The present investigation deals with the transport of glucose into the myocardial cell (a rate limiting step in myocardial glucose utilization), as indicated by the entry and accumulation of galactose-C^{14} following intravenous administration. The effects of digoxin (0.065 mg/kg) and insulin (1 u/kg) were compared. Administration of these drugs to the anesthetized intact dog resulted in a marked increase in the galactose entry and accumulation in the myocardium, thus indicating a marked increase in the glucose transport processes of the myocardium. The similarity of the effects of digoxin and insulin suggest that digoxin augments the metabolism of the myocardium by increasing the transport of glucose into the myocardial cell. **(These studies were aided by Grant # H2354C2, U.S.P.H.S.)**

10 **Pharmacology**

HIGH ENERGY PHOSPHATE COMPOUNDS IN EXPERIMENTAL HEART FAILURE PRODUCED BY COARCTATION OF THE AORTA. M.B. Feinstein (Intr. by J. Belford) State Univ. of N.Y., Brooklyn, N.Y.

Experimental coarctation (EC) of the aorta in guinea pigs, produced cardiac hypertrophy, pulmonary and hepatic congestion and electrocardiographic changes, which resemble the symptoms of congestive heart failure. (EC) animals were sacrificed from one to 18 days after surgically producing aortic coarctation, at which time a portion of the left ventricle was quickly removed under artificial respiration and frozen in liquid nitrogen. Perchloric acid extracts were prepared from these frozen tissue samples and assayed for adenine nucleotides, inorganic and creatine phosphate, and free creatine by the methods of Kalckar (J. biol. Chem. 167:445, 1947), Furchgott and deGubareff (J. biol. Chem. 223:377, 1956) and Eggleton et al. (Biochem. J. 37:526, 1943) respectively. The mean concentrations (in umoles/gm. wet weight) of these compounds in control animals were: ATP 5.5, ADP 0.6, AMP 0.3, IP 3.7, CP 8.5 and free creatine 8.4. In 20 (EC) animals, exhibiting varying degrees of severity of congestive failure, the mean values obtained were: ATP 4.2, ADP 0.4, AMP 0.4, IP 7.8, CP 4.2 and free creatine 6.3. These results indicate that a significant loss of high energy phosphate compounds and total creatine occurs in cardiac failure induced by this experimental method.

070. CARDIOVASCULAR SYSTEM–Cardiac Output

1 **Physiology**

MIXING OF INDICATOR WITH BLOOD IN THE LEFT VENTRICLE. Clorinda Scarpa-Smith (intr. by Philip Dow.) Dept. Physiology, Medical College of Georgia, Augusta.

Past literature offers divers ways of estimating ventricular volume at the end of systole or diastole from dilution of an indicator. All methods assume homogeneous mixing of the indicator with the contents of the ventricle. The desire to use one of these methods dictated a test of the mixing assumption. Open-chest dogs under morphine-pentobarbital or chloralose anesthesia were used. For several seconds, immediately after injection of T-1824 into the left atrium, either integrated or serial samples were collected simultaneously from the root of the aorta and from some part of the ventricle. The sampling systems (needle and polyethylene tubing) were almost identical and of minimal volume. Despite considerable variability, samples from the tip of the ventricle consistently (80-85% of the trials) contained less dye than those drawn from the aorta. This was true of both the integrated samples and the irregular curves determined by the serial samples. The discrepancies were easier to demonstrate when the heart was large and slow than when it was small and fast. It is difficult to reconcile these findings with any picture of homogeneous intraventricular mixing or to use with confidence any method depending on that assumption. (From the Cardiovascular Research Training Program, USPHS Grant HTS 5044.)

2 **Physiology**

ACUTE COMPENSATION IN CARDIAC OUTPUT ON OPENING AN A-V FISTULA. Arthur C. Guyton. Dept. of Physiology and Biophysics, Univ. Med. Center, Jackson, Miss.

Acute A-V fistulae have been prepared in dogs by shunting blood from the two femoral arteries directly back into the right atrium. The maximum blood flow through the fistulae ranged between one-half and two-thirds the total cardiac output. Experiments were carried out in animals under total spinal anesthesia but receiving a nor-epinephrine drip (these animals had normal pressures but no circulatory reflexes) and in animals with an intact nervous system. In both groups acute opening of the fistulae increased the total cardiac output immediately to a value almost sufficient to maintain normal systemic blood flow. The systemic blood flow in the animals without circulatory reflexes fell to about 20 per cent below normal while the blood flow in those animals which had reflexes fell to about 10 per cent below normal. The principal mechanism by which the circulatory reflexes helped to compensate was to increase the mean circulatory pressure which rose in these experiments from an average of 7 mm. Hg to 9 mm. Hg. Transfusion of blood into both groups of animals caused a rise in mean circulatory pressure and was capable of returning the systemic blood flow to completely normal values, thus illustrating the importance of blood volume increase in A-V fistulae as a compensatory process.

3 **Physiology**

EFFECTS OF EXERCISE ON HEART OUTPUT IN NORMAL DOGS. M. D. Bailie*†, Sid Robinson and J. L. Newton*. Indiana University, Bloomington, Ind.

This study was designed to determine by means of the direct Fick method the effect of varying rates of work on the heart output of healthy young dogs. Blood was sampled simultaneously through a polyethylene catheter in the right ventricle and an inlying needle in the left carotid artery. Heart rate was determined from strain gauge pressure recordings. Oxygen consumption was determined by the open circuit method. Oxygen consumption of the dogs was found to be linearly related to rates of work in runs on a treadmill at 5.6, 9.6 and 12.9 km/hr up an 18% grade. Minute volume of the heart, heart rate, stroke volume and arterio-venous oxygen difference were found to increase with oxygen consumption. Heart rate increased from an average of 116 in rest to 260 in the hardest work with a corresponding increase in stroke volume from 29 to 55cc. The average minute volume in the hardest work was 14.3 l./min. A heart rate of 500 would have been required to produce this minute volume without an increase in stroke volume. From these data we have concluded that the increase in stroke volume was a necessary condition for maintenance of the high minute volumes found in exercise. (Supported by a grant from the National Science Foundation) (†N.I.H. Fellow).

4 **Physiology**

VARIABILITY OF CARDIAC OUTPUT AND RESPIRATION OF DOGS DURING PENTOBARBITAL ANESTHESIA. Allan Hemingway. Dept. Physiology, Univ. Calif. School Med., Los Angeles.

Dogs were anesthetized with a single dose of pentobarbital Na, 25 mg/kg, and measurements were made of respiration and cardiac output, before and during the two hours period following induction of anesthesia. Blood concentrations of the anesthetic were determined. Pentobarbital anesthesia causes a fall in respiratory rate and ventilation rate following administration of the anesthetic. A minimum value is reached in 15-35 minutes after which there is a progressive rise. Oxygen consumption rate, arterial and mixed venous blood oxygen contents were determined and cardiac output computed by the Fick procedure. Oxygen consumption rate and A-V (O_2) difference fell in the hour interval following anesthesia but oxygen consumption rate was reduced more than the A-V differences. Cardiac output fell to a value equal to approximately one-half to two-thirds of the pre-anesthesia value and after reaching a minimum value in 15-30 minutes, it rose slowly in the next 90 minutes. A procedure is suggested for reducing variability resulting from anesthesia when pentobarbital Na is used. (Supported by grant H-1243 from the Public Health Service.)

5 **Physiology**

ESTIMATION OF LEFT VENTRICULAR VOLUMES FROM THERMODILUTION CURVES. Charles R. Thorpe* and Fred S. Grodins. Northwestern University Medical School, Chicago, Illinois.

The dilution method of Holt (1954) was modified by using heat as the test agent (Fegler, 1954). In open chest anesthetized dogs, a catheter was introduced into the left ventricle via the left atrial appendage. A thermocouple was passed down a carotid artery to a point just above the aortic valve, and blood temperature was recorded on a Sanborn oscillograph with an overall time constant of 0.15 sec. Following the "instantaneous" injection of 2 ml of cold saline (5°C) into the left ventricle, a thermal dilution curve was recorded from the aortic couple. Provided the couple was very close to the aortic valve, these curves showed the same type of "staircase" descending limb as Holt observed for dye and conductivity curves. Duplicate determinations in each of 16 dogs yielded the following results (mean ± coeff. of variation): cardiac output = 1740 ml/min (± 13.1% within dogs; ± 40.8% between dogs); stroke volume = 10.2 ml (± 13.9% within; ± 45.1% between); ESV/EDV = 0.763 (± 3.5% within; ± 15.6% between); EDV = 44.8 ml (± 22.3% within; ± 28.6% between). This method promises to provide a convenient and useful estimate of both ventricular volumes and cardiac output. (Supported by a research grant, H-1626, from NHI, NIH).

XUM

6 **Physiology**

DETERMINATION OF CARDIAC OUTPUT FOLLOWING A MEAL IN THE UNANESTHETIZED DOG. Edward J. Reininger and Stephen Mutik (intr. by B.D. Burns). Dept. of Physiology, McGill University, Montreal, Canada.

In earlier experiments the effects of feeding on cardiac output and its distribution were shown in anesthetized rats (Reininger & Sapirstein, Science, 126, 1176, 1957 and The Physiologist 1, No. 4, 65, 1958). A redistribution of blood flow to the splanchnic area does not occur at the expense of other areas; all organs received a larger flow which was proportional to the increased cardiac output associated with feeding. This study was undertaken to determine the cardiovascular effects of feeding without the interfering influence of an anesthetic and to determine specific action of protein and fat meals. Six dogs, trained to remain on their sides during an experiment, were used repeatedly. Successive cardiac outputs were determined by the dye dilution technique using BSP. The treatments include: a large protein meal (1 lb. of liver); an isocaloric amount of beef fat (73 grams) and a control series. The cardiac outputs 1, 2 and 4 hours after feeding were compared with the average of two pre-feeding values. A significant increase of 23% and 34% in cardiac output was measured 1 and 2 hours respectively following a liver meal. The fat meal did not cause any significant change in the cardiac output. (Supported by a grant from the Quebec Heart Foundation.)

7 **Physiology**

INFLUENCE OF SYMPATHICOMIMETIC AMINES ON CARDIOVASCULAR RESPONSE TO EXERCISE IN THE DOG. Michael J. Allwood,* Ernst W. Keck,* John T. Shepherd. Mayo Clinic and Mayo Foundation, Rochester, Minnesota.

The indicator-dilution technic was used to measure cardiac output (C.O.) and lung and left heart blood volume (C.B.V.) in intact dogs. During severe exercise, with C.O. up to 10 L./min., stroke volume (S.V.) and C.B.V. increased by 0-15%. Infusion of epinephrine (4-32 μg./min.) at rest caused a 0-70% increase in C.O., slight or moderate increase in S.V. and arterial pressure (B.P.) and slight increase in C.B.V.; with exercise, little further change occurred in S.V., B.P. or C.B.V., and C.O. was similar to that during control exercise. Norepinephrine (4-32 μg./min.) at rest caused bradycardia, little change in C.O., marked increase in S.V. and B.P. and slight increase in C.B.V.; with exercise, little further change occurred in S.V., B.P. or C.B.V., and C.O. was less than during control exercise. Isoproterenol (2-16 μg./min.) at rest caused marked tachycardia and increase in C.O., unchanged S.V., hypotension and a definite fall in C.B.V. With exercise, S.V. did not change further and hypotension persisted; C.B.V. approached and C.O. continued to exceed control values.

8 **Physiology**

SIGNIFICANCE OF CHANGES IN "CENTRAL" BLOOD VOLUME IN MAN DURING EXERCISE. Robert J. Marshall (intr. H. J. C. Swan). Mayo Clinic and Mayo Foundation, Rochester, Minnesota.

Indicator-dilution curves were recorded from the right radial artery following central injections in young men. The mean transit time (M.T.T.) was corrected for delay in the recording system. In the supine position at rest the "central" blood volume (C.B.V.) was 1760 to 2290 ml. During severe leg exercise it increased to as much as 5240 ml. In this circumstance, however, blood flow to the legs is increased 10-fold and that to the arms is reduced. During identical exercise the arterial flow to the right arm was augmented by heating or by reactive hyperemia. The M.T.T. of the resulting curves was greatly reduced and the disappearance slope more steep. The increase in C.B.V. was abolished. Thus the increased C.B.V. during leg exercise is due largely or entirely to alterations in the equitemporal boundaries of its "arterial" component induced by systemic redistribution of flow. Hence when blood is sampled peripherally, conclusions cannot be drawn about changes in lung blood volume. Further, since the disappearance slope is affected by changes in the M.T.T. through systemic arteries, the slope volume is not an index of pulmonary mixing volume.

9 **Physiology**

CARDIAC OUTPUT IN DOGS WITH ACUTE AND CHRONIC COMPLETE ATRIOVENTRICULAR DISSOCIATION: EFFECT OF HEART RATE, TILTING AND PENTOBARBITAL ANESTHESIA. Gerold L. Schiebler, Arthur H. Kitchin and David E. Donald (intr. by Hiram E. Essex). Mayo Clinic, Rochester, Minnesota.

Cardiac output was determined by the indicator-dilution technic, and aortic and right atrial pressures were recorded in 10 dogs with complete atrioventricular dissociation. Studies were performed in the immediate postoperative period in six dogs, and seven experiments with and without anesthesia were conducted in another four trained dogs at 2 to 5 months after operation. Determinations were made at 2 and 5-minute intervals at the dog's idioventricular rate and after the ventricular rate was changed by electrical stimulation to 60, 100, 150 and 200 beats/minute. Observations were repeated with the dog resting in the supine horizontal, 45° head-up and 45° head-down positions. Cardiac outputs were similar over the range of 100 to 200 beats/minute because of rapid adjustments in stroke volume. Values at 60 beats/minute tended to be slightly less (average 11±2.5%) than those at 100 beats/minute and were not significantly different from values obtained at the idioventricular rates, which averaged 52±2 beats/minute. Irrespective of rate, cardiac output values in the 45° head-down position were similar to those in the supine horizontal but were diminished in the 45° head-up position. These effects were similar in the awake and anesthetized states.

10 **Physiology**

DYE-DILUTION CURVES FROM PULMONARY-ARTERY AND AORTIC SAMPLING SITES COMPARED WITH THE SAME CURVES SAMPLED AT THE FEMORAL ARTERY. James B. Bassingthwaighte, Anthony W. T. Edwards (intr. by Howard B. Burchell). Mayo Clinic and Mayo Foundation, Rochester, Minnesota.

A basic assumption in measurement of cardiac output by the Stewart-Hamilton method is that blood flow past the sampling point is constant. In studying ventricular or central blood volumes, sampling from the great vessels near the heart is desirable, but the question arises whether areas of such curves represent the cardiac output. Using measures to eliminate instrumental differences, curves have been recorded simultaneously at femoral-artery and ascending-aorta or pulmonary-artery sampling sites after injection of indocyanine green into the low inferior vena cava (IVC) or superior vena cava (SVC) of anesthetized dogs.

Areas of aortic curves averaged 99.0% (n=16, S.E. of mean = 1.91) of corresponding femoral areas, indicating that sampling from a site of intermittent flow per se did not vitiate estimates of cardiac output. Areas of pulmonary-artery curves averaged 96.0% (n=24, S.E. = 1.66) of femoral areas after IVC injections and 91.1% (n=20, S.E. = 3.66) after SVC injections. These differences could not be shown to be due to delayed development of the color intensity of the dye-protein complex. The results suggest that, when sampling from a site of intermittent flow, the areas are more representative of cardiac output if the indicator is injected farther upstream.

11 **Physiology**

CALIBRATION OF MULTIPLE DYE-DILUTION CURVES IN A SINGLE SUBJECT. Anthony W. T. Edwards,* William F. Sutterer* and Earl H. Wood. Mayo Clinic and Mayo Foundation, Rochester, Minnesota.

In performing multiple dye curves in one subject, problems arise from dye accumulation in the blood. The blood level of indocyanine green in an 18-kg. dog was 4.2 mg./L. after 11 injections of 1.25 mg. during 45 minutes, and 20 mg./L. after also 40 2.5-mg. injections in 3 hours. Because of near-logarithmic diminution in transmitted light with increasing dye concentration, this results in progressive fall in amplitude of the dye curves unless gain is increased. Curves may be calibrated individually, but uniformity of calibration throughout would be very advantageous. If gain is to be increased, the position of electrical zero of the circuit relative to the zero light position is important. In iron-selenium cell or phototube densitometers, indocyanine in blood does not follow Beer's law at high concentrations, but an excellent fit is obtained in range 0-36 mg./L., with this curve approaching an asymptote above the zero light position. If electrical zero is set to coincide with this asymptote, and the base line of each curve is set to the original base line position by altering the gain, adherence to a single calibration curve, that is, near-perfect compensation for background dye, is possible.

12 **Physiology**

CIRCULATORY RESPONSE TO CYTOCHROME OXIDASE INHIBITION IN VIVO. William E. Huckabee. Boston U. School of Med., Boston, Mass.

An increase in cardiac output is caused by low O_2 gas breathing and also by anemia, suggesting that lowered Pa_{O_2} is not an essential part of the circulatory stimulus in the "O_2 sensitive" control system, but lowered P_{O_2} in tissue might be the stimulus. However, a further distinction seems necessary between metabolic effects of lowered O_2, and O_2 concentration itself, which might be remote from the stimulus mechanism by a number of partially independent steps. Blockade of the O_2-cytochrome oxidase step in the tissues induced "hypoxic" effects in metabolic processes while allowing O_2 levels to be in excess, and was effected in intact dogs with I.V. sodium cyanide. All sub-lethal doses produced striking increases in cardiac output and stroke volume and in leg blood flow, despite substantial increases in venous and tissue P_{O_2}. $\dot{V}_{O_2}$ and $\dot{V}_{CO_2}$ were initially depressed but rose again to normal without affecting the circulatory stimulation at any level; anaerobic metabolism was active in both phases. Blood pH was constant at smaller doses of cyanide. Ventricular diastolic pressure fell or remained unchanged; pulmonary artery pressure rose markedly. Pre-treatment with large doses of phentolamine, sufficient to abolish circulatory response to high rates of epinephrine infusion, or of sympatholytic ganglionic blocking agents, did not diminish the response to cyanide administration.

13 **Physiology**

THE SIMULTANEOUS MEASUREMENT OF CARDIAC OUTPUT IN DOGS BY THE USE OF AN ELECTROMAGNETIC FLOW METER AND A TRICARBOCYANINE DYE. W. H. Hardesty,* G.K. Danielson,* and W.S. Blakemore (intr. by J. E. Rhoads). Harrison Dept. Surg. Res., Sch. of Med., U. of Pa., Phila., Pa.

In 8 mongrel dogs, 7.7-12.7 kilograms, 28 simultaneous determinations of cardiac output (CO) were made by the dye dilution (DD) technic using a tricarbocyanine dye ("Cardio-Green") and a non-cannulating square wave electromagnetic flow meter (FM) placed around the isolated main pulmonary artery. The DD values were 89.7% + 4.9% of the FM values. The CO varied from 210 to 1400 cc/minute. The FM and DD values were used only when the calibration curves were essentially linear, the base lines were steady, and the DD curves were not prolonged in duration and flattened to give large errors when replotted on semi-log paper. Previous reports of comparisons between the Fick and the DD methods show correlation without significant systematic differences. The wide application of the FM to the measurement of blood flow in the exposed unopened vessel augments the importance of these comparative simultaneous studies. (Supported by the Southeastern Penna. Chapter of the American Heart Association).

14 **Physiology**

DECLINE IN CARDIAC OUTPUT IN DOGS FOLLOWING CHLORALOSE AND URETHANE ANESTHESIA. H. Page Mauck, Jr., Jack Freund and Reno R. Porter. Med. Coll. of Va., Richmond, Virginia. (Intr. by Leslie E. Edwards).

The behavior of the cardiac output following general anesthesia was studied in 9 mongrel dogs. The animals were anesthetized with chloralose, 75 mgms/kilo. and urethane 750 mgms/kilo. Intravascular catheters were placed in the pulmonary capillary, pulmonary artery, right ventricle and right atrium, and a sound was placed in the carotid artery. Pressure was monitored continuously by a Sanborn Polyviso. Cardiac output was determined by the indicator-dilution method using T-1824 and femoral arterial blood sampling. After a 2-hour period of anesthesia, cardiac output was determined and blood pressure, heart rate and intravascular pressures recorded. The same determinations were repeated 1 hour later. A fall in mean cardiac output from 3.6 L/min. to 3.0 L/min. ($P<.01$) occurred in the 1-hour period between determinations. Heart rate and blood pressure changed insignificantly. Pulmonary capillary, pulmonary artery and right atrial mean pressures fell slightly and end-diastolic pressure did not change. These results, and previous findings by other workers with pentobarbital anesthesia indicate that in any animal experimentation involving cardiac output studies cognizance must be taken of the declining output curve following intravenous anesthesia. (Supported by a grant from the Virginia Heart Association.)

15 **Physiology**

EFFECT OF INCREASING AORTIC PRESSURE ON THE PERFORMANCE CHARACTERISTICS OF THE LEFT VENTRICLE. J.H.Mitchell*, J.P.Gilmore, R.J.Linden*, S.J.Sarnoff (intr.by Dr.R.Daggs). Lab.Cardiovascular Physiol.,NHI, Bethesda, Md.

Increasing the resistance to ventricular ejection in the isolated supported heart preparation produced cardiodynamic alterations closely resembling the positive inotropic effect on the ventricle caused by catechol amines. If the aortic pressure was suddenly increased and maintained at a higher level, the following findings were noted: (1) the left ventricular pulse contour revealed a more rapid development of tension, a more rapid relaxation, a decreased systolic ejection time, and a longer diastole for a given stroke volume and heart rate, (2) an increased external stroke work from any given left ventricular end diastolic pressure, and (3) an increased production of power (external stroke work per systolic second) from any given left ventricular end diastolic pressure. For any given level of work, during a higher aortic pressure, coronary flow is increased, A-V O_2 difference is decreased, and the O_2 saturation of coronary venous blood is increased. Studies including the simultaneous recording of arterial and coronary venous blood pO_2 and pH will be discussed in relation to the concept of autoregulation of the performance characteristics of the heart.

16 **Physiology**

THE CAROTIDO-VAGO-ATRIAL AND CAROTIDO-SYMPATHO-ATRIAL REFLEXES J.P.Gilmore, J.H.Mitchell*, S.J.Sarnoff. Lab. Cardiovascular Physiol., NHI, Bethesda, Md.

It was previously observed that cardiac sympathetic nerve stimulation increased and vagal stimulation decreased atrial contractility. The present experiments were conducted to ascertain whether carotid sinus nerve stimulation can reflexly excite these efferent pathways. In order to observe more readily the induced changes in atrial systole undisturbed by contiguous ventricular activity, the experiments were carried out in dogs with surgically induced heart block. The data obtained demonstrate that (a) carotid sinus hypertension or stimulation of the carotid sinus nerves decreases the vigor of atrial contraction via increased vagal and decreased sympathetic activity; the vagal effect is blocked by atropine, (b) carotid sinus hypotension increases the vigor of atrial contraction via decreased vagal and increased sympathetic activity, (c) the operation of these reflex pathways substantially alters the vigor of atrial systole, the consequent contribution of the atria to ventricular end diastolic pressure and fiber length and thus, the external stroke work produced by the ventricle.

17 **Physiology**

EFFECTS OF STELLATE GANGLION STIMULATION ON THE PERFORMANCE OF THE DOG HEART LUNG PREPARATION. L. J. O'Brien (intr. by R. S. Alexander). Department of Physiology, Albany Medical College, Albany, New York.

Observations were made on the changes in cardiac function in the dog heart lung preparation brought about by stimulation of the left stellate ganglion and were compared with observations obtained when the heart was driven by an artificial pacemaker. During left stellate ganglion stimulation, cardiac output, stroke volume, and heart rate were all enhanced; left ventricular diastolic pressure fell slightly while left ventricular systolic pressure showed no significant change. Pressure in the venous reservoir was held constant throughout the period of observation. When heart rates obtained during stellate stimulation were approximated by right atrial stimulation, there was no change in cardiac output and left ventricular systolic pressure, a slight increase in left ventricular diastolic pressure, and approximately a 20 per cent decrease in stroke volume. The 20 per cent reduction in stroke volume produced by the artificial pacemaker conforms with the Starling relationship and relates to decreased filling time; adrenergic stimulation counterbalanced this effect to the point that a 25 per cent increase in stroke volume was observed. (Supported by a grant from the National Heart Institute.)

XUM

18 **Physiology**

THE EFFECT OF HEART RATE ON AORTIC INSUFFICIENCY. Homer R. Warner and Alan F. Toronto*. Latter-Day Saints Hosp. & Univ. of Utah Salt Lake City, Utah.

Studies were performed on three patients with aortic insufficiency. From simultaneous recordings of dye concentration as a function of time in left radial and femoral artery blood following injections of dye late in systole into the descending aorta at measured distances from the origin of the left subclavian artery, the maximum distance (D) over which backflow, due to the aortic insufficiency, carried the dye with each diastole was determined (Cir.Res.6:29,1958). The product of cross-sectional area of the aorta (estimated), heart rate, and D is used as a measure of insufficiency. Dye was also injected into the pulmonary artery for determination of net cardiac output. Efficiency of left ventricular ejection is here defined as the ratio of net cardiac output to the sum of net cardiac output and insufficiency. All measurements were performed at two heart rates, the higher rate being produced by electrical stimulation of the right atrium through a metal-tipped catheter forced against the right atrial wall. At the faster heart rates (120,120,105) all three patients showed less insufficiency (-39,-63,-42%) and higher efficiency (+14,+16,+18%) than during the control rates (78,84,60).

19 **Pharmacology**

MECHANISM OF CARDIAC RESPONSES TO CAROTID OCCLUSION AND TO INJECTION OF PRESSOR DRUGS. C. Polosa and G. Rossi (intr. by R. P. Ahlquist.) Depts. Physiology, Pharmacology and Surgery, Med. Coll. of Ga., Augusta, Ga.

Regulation of cardiac output during carotid occlusion can result from the interplay of many factors. Arteriolar constriction with increased peripheral resistance, venous constriction with increased venous return, and changes in heart rate and myocardial contractility are the factors presumably involved. Experiments have been undertaken in anesthetized dogs to analyse the role played by each of these factors. These include the comparison of the pressor response to carotid occlusion before and after the beta adrenergic receptor blocking agent dichloroisoproterenol, and the determination of the cardiac output by the pulse contour method and by an electromagnetic flowmeter. The pressure-flow measurements seem to indicate an increase in TPR as the predominant factor in the carotid occlusion pressor reflex. Responses to intravenous injections of various doses of catecholamines are being studied by the same methods. (From the Cardiovascular Research and Training Program, HTS-5044, NHI, USPHS, and Am. Heart Ass.)

071. CARDIOVASCULAR SYSTEM—Cardiovascular Drugs

1 **Physiology**

THE CARDIOVASCULAR PHARMACOLOGY OF A HYPOTENSIVE DRUG WITH DIRECT CARDIAC EFFECTS. Kenneth I. Colville*, Martin M. Jacobson* and Charles H. Ellis. Wellcome Research Laboratories, Tuckahoe, N.Y.

B.W. 57-301, 2-amino-4-(N'-methyl-N-piperazino)-5-(4'-chlorophenylmercapto)-pyrimidine hydrochloride, produces in dogs anesthetized with pentobarbital a prolonged fall in arterial pressure accompanied by a decrease in heart rate. Neither effect is blocked by premedication with atropine or by bilateral vagotomy. Peripheral vasodilatation occurs following the drug and also a reduction in the force of ventricular contraction (Walton gauge). Cardiac dilatation has been demonstrated following B.W. 57-301 by cardiometric and cinematographic methods. A rise in venous pressure does not occur unless lethal doses are given. In the Langendorff perfused rabbit heart the drug causes a decreased force of cardiac contraction and with larger doses a slowing of heart rate. The drug lacks adrenergic blocking or ganglionic blocking properties and is not a cholinomimetic.

2 **Physiology**

EFFECTS OF ISCHEMIA AND HYALURONIDASE UPON MYOCARDIAL WATER, SERUM ALBUMIN, AND ERYTHROCYTE CONTENT. Jorge Martins de Oliveira* and Matthew N. Levy. St. Vincent Charity Hosp., Cleveland, Ohio.

The left circumflex coronary artery was ligated in a series of anesthetized dogs. Five hours later, the content of water, I^{131}-labeled serum albumin, and Cr^{51}-labeled erythrocytes were compared in an ischemic region (post papillary muscle) and in a normal zone (anterior superior left ventricle). The red cell content of each zone was virtually identical, but the water and serum albumin contents were significantly higher in the ischemic region, probably consequent to an increased capillary permeability. Hourly intravenous injections of large doses of hyaluronidase do not affect the red cell or albumin content of the ischemic zone appreciably, but effectively prevent the increased water content. This is probably a combined effect of the action of this enzyme as a spreading factor and as a lymphagogue.

3 **Pharmacology**

OBSERVATIONS ON THE HYPOTENSIVE ACTION OF PHENOTHIAZINES IN THE UNANESTHETIZED DOG. M. Porter*, H. L. Froehlich*, D. Arbakow*, J. N. Spencer and H. Wendel. Smith Kline & French Laboratories, Philadelphia, Pa.

In the anesthetized animal there is little difference between the hypotensive effects of 'Thorazine' (Tz), 'Compazine' (Cz), and 'Stelazine' (Sz), but clinically, hypotension is most frequently encountered with Tz. In view of this apparent discrepancy, the comparative hypotensive activity of the three drugs was investigated in the unanesthetized dog. Tz on i.v. or p.o. administration resulted in a marked decrease in blood pressure lasting 24 hours or more. The degree of hypotension on i.v. injection was comparable to that observed in the anesthetized animal. Cz in small i.v. doses produced transient hypotension but within 1 or 2 minutes the blood pressure had returned to the preinjection level. Larger i.v. doses resulted in a sustained hypotension but less marked than that seen with Tz. The blood pressure was not altered by Cz orally in doses as large as 20 mg/kg. Sz in small i.v. doses in some dogs produced an increase in blood pressure; in others it produced a transient hypotension comparable to that observed following injection of Cz. Larger doses resulted in a slight hypotension of moderate duration. Orally, large doses of Sz produced a decrease in blood pressure of questionable significance. These results and those obtained in other studies will be discussed.

4 **Pharmacology**

STUDIES ON MORPHINE HYPERGLYCEMIA. M. Vassalle (intr. by V. Sydow.) Depts. Physiology and Pharmacology, Med. Coll. of Ga., Augusta, Ga.

Relationship between morphine hyperglycemia and the adrenal glands was studied in unanesthetised normal and chronically adrenalectomized dogs. Blood samples for sugar and hematocrit were taken every 20 min. Control samples (3-6) showed no change; then morphine sulfate (10 mg/kg) was administered subcutaneously. This elicited a marked rise in blood sugar (up to 185% of control) that reached its maximum within 1 hr. Venous hematocrit rose. After adrenalectomy morphine elicited in the same dog a small increase in blood sugar, although in one case it was as much as 64% of control. Dihydroergotamine (0.2 mg/kg) intravenously, markedly reduced morphine hyperglycemia in normal dogs and abolished it in the adrenalectomised dogs. Atropine sulfate (1 mg/kg) produced, if anything, an increase in the morphine hyperglycemia. Release of epinephrine appears to play a main role in morphine hyperglycemia. Adrenergic blockade prevents any rise in blood sugar in adrenalectomized animals. Atropine, which in small doses inhibits the central effects of acetylcholine, did not prevent the morphine hyperglycemia. It seems, therefore, that epinephrine release is not mediated through the anticholinesterase activity of morphine in the central nervous system. (Supported by training grants HTS-5044, NHI, USPHS, and AHA.)

5 **Pharmacology**

SYSTEMIC AND CORONARY HEMODYNAMIC EFFECTS OF ANSIV (1-(2-METHOXYPHENYL)-4-(3-METHOXYPROPYL)-PIPERAZINE PHOSPHATE). (ABBOTT H.T. 1479). G. O. Rowe, C. A. Castillo*, S. Afonso*, H. P. Gurtner*, C. J. Chelius* and C. W. Crumpton. Dept. of Med. and Cardiopulmonary Res. Lab., U. of Wis., Madison 6, Wisconsin.

Acute systemic and coronary hemodynamic effects of Ansiv have been measured in 10 dogs, anesthetized with morphine and Dial-Urethane. Determinations of cardiac output were done by the Fick principle and coronary blood flow by the N_2O method. The animals were studied before and after 0.5 to 1 mg./kg. of Ansiv intravenously. Cardiac rate increased by 52.9% ($p<0.01$). Mean blood pressures decreased in the systemic artery by 17.8% ($p<0.01$) in the pulmonary artery by 13.3% ($p<0.05$) and in the right atrium in 9 of 10 dogs. Minute volume of respiration, O_2 consumption, CO_2 liberation and body R. Q. increased significantly. Coronary sinus O_2 content decreased significantly with widening of the arterial-coronary sinus difference for both O_2 and CO_2. Cardiac output did not change significantly, but total peripheral resistance decreased 27.9% ($p<0.02$). Coronary blood flow, cardiac O_2 usage, and cardiac efficiency did not change significantly. Ansiv appears to lower blood pressure through peripheral vasodilatation without a significant change in cardiac output as opposed to ganglion blocking drugs which lower blood pressure by decreasing cardiac output.

6 **Pharmacology**

SYMPATHETIC BLOCKING AGENT(BRETYLIUM) ON CARDIOVASCULAR RESPONSES. Domingo M.Aviado and Abdur Dil.* Dept. Pharmacology, Univ. of Penna. Schools of Medicine, Phila., Penna.

This new agent (N-o-bromobenzyl-N-ethyl:N:N-dimethylammonium p-toluene sulfonate)was discovered by Boura et al to block sympathetic nerves without antagonizing the effects of released or injected catecholamines (Lancet 2:17, 1959). In anesthetized dogs, the intravenous injection of 2.5 to 20 mg/kg of bretylium caused variable effects on aortic and pulmonary arterial blood pressures. These were accompanied by transient tachycardia, increased force of myocardial contraction and increased pulmonary blood flow. A combination of an injection of bretylium(to block sympathetic nerves) and adrenalectomy was required to block completely the stimulatory responses to the following: a)common carotid occlusion; b)increased intracranial pressure; c)inhalation of 5% oxygen; d)inhalation of 5% carbon dioxide in air; and e) intravenous injection of DMPP. Procedure e) is predominantly dependent on adrenal medullary mechanism, procedure d) on sympathetic nerves, whereas a), b) and c) are dependent on both.

7 **Pharmacology**

ETHYLENEDIAMINE, A TOOL TO UNMASK CARDIAC CHANGES IN THE TURTLE RESULTING FROM ENVIRONMENTAL FACTORS. Harold F. Hardman and Pojana Bukhamana*, Univ. of Mich., Ann Arbor, Michigan.

Ethylenediamine produces a negative inotropic action in isolated turtle hearts tested during winter months. In summer months this drug produces a positive inotropic response under the same laboratory conditions present during the winter months. Summer hearts may be converted to winter hearts, as evaluated by the response to ethylenediamine, by storing the intact turtle at 5° C. for two weeks. It has been established that ethylenediamine may exist in three different physical states over the pH range of 6.5-8.5. The three physical states are referred to as the divalent cation, monovalent cation and the unionized form. The negative and positive inotropic actions of the drug can be correlated only with the monovalent cationic form. Preliminary evidence suggests that ethylenediamine exerts its effects on the turtle heart through the release of histamine. This suggestion is supported by the observation that exogenous histamine produces a negative inotropic action in winter turtle hearts and a positive inotropic action in summer turtle hearts. (Supported by the Michigan Heart Association.)

8 **Pharmacology**

THE REVERSAL OF OUABAIN INDUCED CARDIAC ARRHYTHMIAS BY DICHLOROISOPROTERENOL. Benedict R. Lucchesi. (intr. by H.F. Hardman), Univ. of Michigan, Ann Arbor, Mich.

Recent studies in our department concerning the action of dichloroisoproterenol (DCI, Lilly Compound 20522) upon conduction and refractory periods in the dog heart-lung preparation led us to suspect that this compound might prove of value in antagonizing the cardiac arrhythmias resulting from overdosage with the cardiac glycosides. Studies were conducted in the pentobarbital anesthetized dog in which cardiac arrhythmias were produced by the administration of ouabain, 50 ug./kg. The cardiac arrhythmias were rapidly antagonized and normal sinus rhythm established following the administration of DCI 5 mg/kg. Similar results were obtained in the unanesthetized dog. The isolated perfused rabbit heart (Langendorf) was utilized in an attempt to study the mechanism by which DCI exerts its action. Arrhythmias were produced by the addition of ouabain to the perfusion solution. The cardiac disturbances were rapidly and permanently reversed upon the addition of DCI to the perfusion solution along with the glycoside.
(Supported by the Michigan Heart Association.)

9 **Pharmacology**

EFFECT OF PHENAZOCINE HYDROBROMIDE ON DIURESIS. Bennett P. Lustgarten*, Solomon Fisch*, and Arthur C. DeGraff. Veterans Administration Hospital, Bronx, New York.

Morphine and meperidine inhibit water diuresis in animals, but this effect is not found consistently in saline diuresis. In man, Ferrer and Sokoloff noted these same findings in some normal subjects and in patients with congestive heart failure who were subjected to water and mercurial diuresis respectively. This present study is concerned with a comparison of phenazocine and morphine to determine if the former affects diuresis. Water and mercurial diureses were induced in normals and in patients with congestive failure respectively. An undisturbed diuresis for each patient constituted the control run. Morphine or phenazocine were then given on different days to these same subjects after diuresis was established. Fractional urinary volumes and electrolyte excretions were measured in each run. Results demonstrated that both morphine and phenazocine produced an inhibition of both types of diuresis. It is postulated that phenazocine, like morphine, inhibits diuresis by increasing the output of anti-diuretic hormone.

10 **Pharmacology**

PHARMACOLOGICAL PROPERTIES OF A NEW STEROID ALKALOID (SU-5916) ISOLATED FROM CONOPHARYNGIA PACHYSIPHON. R. Rutledge,* W. E. Barrett and A. J. Plummer. Research Dept., CIBA Pharmaceutical Products Inc., Summit, N. J.

An extract of the roots of Conopharyngia Pachysiphon was found to possess marked hypotensive activity. Recently, Dickel, Lucas and MacPhillamy described the isolation of the active material present in this extract. The active compound was found to be 20 α-amino-3β-hydroxy-5-pregnene-β-D-glucoside (Su-5916). Su-5916 was found to possess marked hypotension when tested in the anesthetized dog. Intravenous doses of 50 γ/kg to 1.0 mg/kg produced a marked and sustained hypotensive response. Su-5916 did not exhibit any adrenolytic properties but instead tended to increase the response elicited by injected epinephrine; it produced no significant alteration in the response produced by serotonin. The drug did not exhibit good oral hypotensive activity. Doses of 10.0 mg/kg injected directly into the small intestine of the anesthetized dog produced a 68 mm decline in the mean arterial blood pressure with recovery to essentially the control level in thirty minutes. Other aspects of the pharmacology of Su-5916 will be presented.

XUM

11 *Pharmacology*

INTERACTIONS OF DIBENZYLINE AND DCI ON THE CARDIOVASCULAR SYSTEM AND NICTITATING MEMBRANE. L.G. Eltherington* and A.Horita. University of Washington School of Medicine, Seattle 5, Wash.

Dibenzyline has been regarded classically as an irreversible adrenergic blocking agent of excitatory (alpha) receptors in the vasculature and nictitating membrane. We have observed that after blockade of the vasculature alpha receptors by Dibenzyline, benzodioxane or dihydroergotamine, subsequent injections of dichloroisoproterenol (DCI) will cause a return of the epinephrine pressor response from 50 to 100 per cent of control values. This drug interaction is true as regards the blood pressure of both anesthetized dogs and cats. The smooth muscle of the cat nictitating membrane however, does not react in the same manner. Once an adrenergic blockade has been established with Dibenzyline, DCI produces no return of membrane contraction after epinephrine. At this time other agents such as acetylcholine, are still capable of stimulating the membrane. Thus it may be that while adrenergic blocking agents and DCI produce an effect on both excitatory and inhibitory (beta) receptors of the vasculature, the smooth muscle of the nictitating membrane does not react in a similar manner.

12 *Pharmacology*

MECHANISM OF ACTION OF MEPHENTERMINE. Charles R. Swaine,* Johanna Perlmutter* and Sydney Ellis. Dept. of Pharmacology, Woman's Medical College of Pennsylvania, Philadelphia.

Our analysis of the mechanism of action of mephentermine (Wyamine) supplements published evidence to indicate that cardiac stimulation is the major factor causing the pressor effect and that the myocardial action is an indirect effect acting through the release of catechol amines. In dogs, DCI (which inhibits cardiac, but not vasoconstrictor, action of sympathomimetic amines) antagonized the pressor effect of mephentermine. The fact that pretreatment with reserpine or cocaine prevented the pressor action of mephentermine suggests an indirect action dependent upon availability and release of catechol amines. Direct evidence for such a release was obtained in a Langendorff cat heart preparation which superfused a Magnus preparation of rabbit jejunum. In this preparation mephentermine increased the heart rate and the perfusate from the heart relaxed the intestine. Mephentermine applied directly to the superfused intestine produced no effect or stimulation. When the cat was pretreated with reserpine, mephentermine did not stimulate the heart nor cause relaxation of the intestine. However, after the reserpinized heart was perfused with norepinephrine and allowed to recover, mephentermine then stimulated the heart and caused the release of a substance which relaxed the intestine. (Supported in part by grants from Wyeth.)

13 *Pharmacology*

EFFECTS OF QUINIDINE ON THE FUNCTION OF THE NORMOTHERMIC AND HYPOTHERMIC HEART. E. Kent Carney, John Ross, Jr., and Theodore Cooper (intr. by Albert Sjoerdsma). National Heart Institute, Bethesda, Md.

Recent interest in the use of large doses of quinidine for the prevention of arrhythmia during hypothermia prompted the present investigation of the effect of quinidine on ventricular function. In 6 dogs the relationship between left ventricular filling pressure and stroke work was determined during the control state and again following quinidine administration. In each animal quinidine (30 mg./Kg.) produced marked depression of the ventricular function curve. Subsequent hypothermia (27°-29°C) resulted in elevation of the curve toward the control level. However, after rewarming, the depression recurred. In 16 dogs, myocardial contractile force, as measured with a strain gauge arch, was depressed to 55% of control levels by the same dose of quinidine. Fifty-six percent of these dogs survived subsequent hypothermia and rewarming. In 3 dogs pretreated with ouabain, the depression of contractile force with rewarming was prevented. In 4 dogs on cardiopulmonary bypass quinidine produced a 23% to 80% fall in arterial pressure at constant perfusion rates. It is concluded that quinidine in large doses depresses cardiac function, and that hypothermia mitigates this depression in part.

14 *Pharmacology*

CORONARY DILATOR RESPONSES FOLLOWING RESERPINE IN THE ISOLATED RABBIT HEART. K.I. Melville, Dept. of Pharmacology, McGill Univ., Montreal, Canada.

Coronary flow and heart action changes have been studied in the isolated rabbit heart perfused by the method previously described (J. Pharmacol., 99, 3, 1950) following reserpine pretreatment (5 mg/kg I.V. 16 to 24 hrs. before excision) using both normal and 'fat-fed' rabbits, i.e. after addition of 2% cholesterol and 6% corn oil to the diet for 4 to 6 mos. (Am. J. Cardiol., 4, 391, 1959). Following injections of nitroglycerin, aminotrate and papaverine (0.2 to 1 mg. each) and aminophylline (1 and 5 mg.), changes were recorded at 1, 2 and 5 mins. in groups of 5 animals. Ach. (10 µg.) and nicotine (0.5 mg.) were injected to test degree of reserpinization. After reserpine there was invariably a relative decreased mean coronary flow and slowing of the heart during the preinjection periods, but the dilator responses to all agents were significantly augmented in both groups. Loss of catecholamines from the heart therefore appears to increase coronary vasodilator sensitivity. This phenomenon might be involved in the favorable action of reserpine in angina pectoris. The problem is being further studied. (Supported by a Quebec Health Grant)

15 *Pharmacology*

ADRENAL CORTICAL STEROIDS AS DIURETICS IN PATIENTS WITH EDEMA OF HEPATIC AND CARDIAC ORIGIN. Harry Gold, Nathaniel T. Kwit, Argyrios J. Golfinos*, Charles R. Messeloff*, Milton L. Kramer*, Elizabeth A Goessel*, and John C. Weaver*. Dept. of Pharmacology, Cornell Univ. Med. College, Hosp. for Joint Diseases, Beth Israel Hosp. and Lincoln Hosp. New York.

It has long been known that some hormones of the adrenal cortex promote retention of sodium and produce edema. More recent experience shows that they may also produce diuresis and abolish edema in some cases. Renewed interest in the diuretic action of the steroids was aroused by experiments with synthetic spirolactones which were shown to reverse the action of aldosterone, a result postulated as a form of competitive inhibition. In previous reports to the Society, we described a new method for comparison of diuretic agents in congestive failure and presented evidence to the effect that an antidiuretic (AD) reaction is an integral part of the diuretic drug action (D), the net response representing a D-AD phenomenon. We have now applied this method to several corticoids, SC9420 (Aldactone), U7913, U19147, prednisone (Meticorten), methylprednisolone (Medrol), and hydrocortisone (Cortef). They all produce diuretic and antidiuretic effects, and both effects may be explained by the D-AD phenomenon as in the case of the conventional diuretics.

072. CARDIOVASCULAR SYSTEM—CV Drugs—Glycosides

1 *Physiology*

THE PATHWAY OF ELIMINATION OF ACETYL STROPHANTHIDIN (AcS) IN DOGS. A.H.Ryan, G.Engler* and H.Wasserman.* The Chicago Medical School, Chicago, Illinois.

Control determinations were made under pentobarbital anesthesia, of the amounts of AcS tolerated and eliminated in periods of 3 to 4 hrs. by giving, at regular intervals, injections of AcS to an endpoint of extrasystoles. Approximately one week later when the renal arteries were ligated (4 dogs), as much AcS was tolerated as on control days; mean 0.6527 ± S.E. 0.0690 mg/kg, control mean 0.6611 ± S.E. 0.0640 mg/kg. When the gastrointestinal (G.I.) arteries and portal vein were occluded, the renal arteries remaining patent (5 dogs), none emerged from the first episode of extrasystoles produced with AcS and died within 3 hrs.; mean L.D. 0.0633 ± S.E. 0.0044 mg/kg. With G.I. and renal vessels occluded (5 dogs) mean L.D. was 0.0859 ± S.E. 0.0170 mg/kg. Snares were placed loosely around the G.I. vessels (5 dogs). Extrasystoles were then produced with AcS. Three minutes after termination of extrasystoles, the G.I. vessels were occluded by tightening the snares, the renal arteries remaining patent. Extrasystoles returned, indicating redistribution of the drug, and continued until the snares were released or until death. The results appear to indicate that AcS is eliminated entirely by the liver and that the drug is loosely bound in the tissues and is redistributed as concentration gradients occur between the tissues and the circulating blood.

2 **Biochemistry**

MODE OF ACTION OF LEVANSUCRASE. Frank Eisenberg, Jr. and Shlomo Hestrin*. National Institutes of Health, Bethesda, Md.

The nature of the donor (raffinose) moiety transferred by levansucrase has been studied at high glucose concentration, a condition which largely suppresses levan formation and which favors the reversible synthesis of sucrose, according to the equation: raffinose + glucose ⟷ melibiose + sucrose, [Hestrin et al., J.Am.Chem.Soc., 77, 6710(1955)]. Glucose-1-O^{18} (1.2 at. % excess O^{18}) was incubated for 90 min. with raffinose and a cell-free extract of Aerobacter levanicum at pH 5.4. Following alcohol precipitation to remove small amounts of levan the incubation mixture was resolved by descending chromatography on sheets of Whatman 3MM in BuOH/HOAc/H_2O, 4/1/5, (upper phase). The sucrose bands, free of raffinose but contaminated by glucose and melibiose, were eluted, combined, and rechromatographed. The resulting pure sucrose bands were eluted, combined, and acetylated to yield sucrose octaacetate, which was recrystallized 3 times from aqueous ethanol. Samples from each recrystallization were taken for m.p. and O^{18} assay. M.p. was constant at 84-86° and O^{18} abundance was constant at 0.40 at.% excess, theoretical for the reaction: mel-O-fru (raffinose) + glu-1-O^{18} ⟷ mel + glu-O^{18}-fru (sucrose, as octaacetate). It is concluded that in levansucrase catalysis the fructosyl (fr) rather than the fructosido (fr~) moiety is transferred: fr~R + enz ⟷ fr~enz + R.

3 **Pharmacology**

EFFECTS OF OUABAIN ON K AND NA CONCENTRATIONS IN RESTING RABBIT AURICLES. P. N. Witt and R. S. Tuttle*. Dept. of Pharmacology, State Univ. of N.Y., Upstate Medical Center, Syracuse, N. Y.

A relationship was sought between dose of cardiac glycoside and its K depleting effect. In control experiments left rabbit auricles incubated for 2 hours at 37°C in Tyrode's solution gradually lost K (31%) and gained Na (202%) as determined in the digests by flame photometry. Ouabain 10^{-7} produced, in beating auricles, a steady increase in contractility. In resting auricles it diminished the 2 hour K loss to 13% while Na concentration was not above controls. 10^{-5} ouabain which produced failure in beating auricles in 30 minutes decreased intracellular K concentration in 2 hours 54% and increased intracellular Na 202%. These results can be interpreted as meaning that a low or "therapeutic" glycoside concentration prevents K loss which is observed under control experimental conditions. On the other hand concentrations of ouabain which are toxic deplete the auricles of K and allow Na entry into the cells. These effects of different concentrations of ouabain may explain discrepancies in the literature. (Supported by a grant from the American Heart Association).

4 **Pharmacology**

FREQUENCY-FORCE RELATIONSHIPS IN RABBIT MYOCARDIUM AND THE ACTION OF OUABAIN. R. S. Tuttle (intr. by A. Farah), SUNY, Upstate Medical Center, Syracuse, N. Y.

The frequency-force relationships in the rabbit auricle could be altered by different concentrations of ouabain. Depending on whether ouabain potentiated or abolished the frequency-force relationships, the terms "toxic" or "therapeutic" could be ascribed to the concentration of ouabain used. The "S"-shaped curve and post-stimulation potentiation (PSP) were studied in isolated left rabbit auricles. They were incubated in oxygenated Tyrode's solution at 37.5°C, stimulated and tension recorded. Our results suggested that the "S" curve as a representative of a frequency-force phenomena, was a physiological relationship since it was present in fresh tissues and absent in depressed tissues. Ouabain had different effects of the "S"-shaped curve and PSP. Low concentrations (10^{-7}M) potentiated the "S" curve and PSP while high concentrations (10^{-6}M) abolished the "S"-shaped curve and reduced PSP. In those preparations where the "S" curve was absent or had been depressed ouabain in concentrations of 5X 10^{-8}M caused a reappearance of the "S"-shaped curve and increased PSP. The results suggest that ouabain has an action on the mechanisms responsible for PSP and the "S" curve and it is postulated that the therapeutic action of ouabain on contractility is related to the potentiating mechanisms under study.

5 **Pharmacology**

RATIO OF CARDIAC TO EMETIC ACTIVITY OF THREE DIGITALIS MATERIALS, DIGITOXIN (CHRYSTODIGIN), DIGOXIN (LANOXIN), AND AMORPHOUS GITALIN (GITALIGIN) IN AMBULANT CARDIAC PATIENTS (PRELIMINARY REPORT). Nathaniel T. Kwit, Harry Gold, Argyrios J. Golfinos*, Elizabeth A. Goessel*, and John H. Hughes*. Dept. Pharmacology, Cornell Univ. Med. College, Hosp. for Joint Diseases, and Beth Israel Hosp. New York.

The view that the ratio of cardiac to emetic activity is not the same for different digitalis glycosides, stressed for digitoxin by warnings that its use is more likely to lead to toxic cardiac effects because it is less likely to issue the gastrointestinal signal, is not supported by valid evidence. Experiences fail to eliminate chance or discriminate between local and systemic emesis. We examined the problem in cardiac patients with relatively stable normal T-waves in the electrocardiogram, using as criteria T-wave inversion for cardiac action and for emetic action, unequivocal impairment of appetite, nausea, or vomiting. The plan called for a daily dose of the drug sufficiently small to avoid local emetic action but adequate for cumulation slow enough to reveal sequential events, a daily electrocardiogram, comparison of all drugs in each patient, several weeks between courses, randomization of courses, and "double-blind" method for interpretation. The preliminary results do not support the current opinion.

6 **Pharmacology**

DEPRESSION OF RESPIRATION RATE DURING DIGITOXIN INTOXICATION IN DOGS AND CATS. T. F. Redick* and P. L. McLain. Dept. of Physiol. & Pharmacol., Univ. of Pgh. School of Med., Pgh., Pa.

Digitoxin (.02 mg/kg) was given i.v. every 5 min. until death to a series of dogs and cats. All animals were under light ether anesthesia. The respiration rates, blood pressures and heart rates were recorded after each dose of digitoxin. Death occurred at an average of 14 doses for cats and 18 doses for dogs. The respiration rate was stable in both series until dose 9. The average respiration rate in the dog series was 70/min. until dose 9 after which it fell rather sharply decreasing to 25/min. at dose 18. The average respiration rate in the cat series was 45/min. until dose 9 and fell after dose 9 to about the same level of 25/min. at dose 14. The respiratory depression did not seem to be directly related to changes in circulation. The mean blood pressure in the dog series gradually rose from a control of 130 to 142 mm Hg. In the cat series the mean blood pressure fell from 122 to 65 mm Hg. prior to terminal fibrillation. The respiration rate started to decrease 2 doses before the average point where irreversible heart arrhythmias occurred. Digitoxin bradycardia, occurring in both dogs and cats, progressed to maximum while respiration rate remained stable at control levels. Thus digitoxin has a depressant effect on the respiratory rate which cannot be interpreted in terms of circulation.

073. CARDIOVASCULAR SYSTEM—CV Drugs—Vasoconstrictors

1 **Pharmacology**

THE EFFECT OF METHYLPHENIDATE UPON LEVARTERENOL BLOOD LEVELS DURING A CONSTANT INFUSION OF LEVARTERENOL. Marvin E. Rosenthale* and Joseph R. Di Palma. Hahnemann Med. Col. Phila

It has been demonstrated by Maxwell and Plummer and also Ivey, that an injection of methylphenidate potentiates the pressor effect of levarterenol. Levarterenol plasma levels in dogs were subsequently determined before and after methylphenidate in order to investigate the mechanism of this potentiating effect. A modification of the trihydroxyindole fluorometric method was employed, for the determination of plasma catechol amine. Levarterenol was administered by a constant infusion pump. The infusion rate was gradually increased over a 1-2 hr. period, until tolerance was obtained. At this time a Rt. atrial sample was taken, 0.3mg/Kg. methylphenidate was injected into the L. jugular vein and another blood sample was obtained one min. after termination of the injection. This is the time required for the maximum pressor effect of methylphenidate to occur. The levarterenol plasma level after methylphenidate rose 30-50% above its control. This also occured in adrenalectomized and splenectomized animals. Statistical comparison with saline control data, showed this increase to be significant. It is concluded that methylphenidate acts as a releaser of stored levarterenol.

XUM

074. CARDIOVASCULAR SYSTEM—CV Drugs–Vasodilators

2 *Pharmacology*

FURTHER STUDIES ON THE VASOPRESSOR POLYPEPTIDE SUBSTANCE A. Edward J. Walaszek and C. G. Huggins*, Dept. of Pharmacology, Univ. of Kansas Medical School, Kansas City, Kansas.

Substance A is a vasopressor polypeptide which is obtained by incubating fraction IV-4 of plasma protein with α-amylase. It is similar to angiotensin in its pharmacological properties. Standard preparations of substance A were found to contain approximately 9% of bradykinin-like activity as assayed on the isolated rat duodenum. This would seem to indicate that small amounts of plasma kinins are also produced during the incubation of the plasma proteins with α-amylase preparations. Chromatography on carboxymethyl cellulose columns permits the purification of the pressor polypeptide substance A. In this way the pressor activity can be separated from plasma kinin activity. It has been determined by parallel assays on guinea pig ileum and rat uterus and chromatographic separation that the purified substance A is not identical with hog angiotensin decapeptide (hypertensin I). The relationship of substance A to the angiotensin octapeptide (hypertensin II) is not known at the present time. The purified substance A does not exhibit tachyphylaxis to its pressor response on dog blood pressure. Unlike angiotensin, substance A can be produced from heat-treated substrate.

3 *Pharmacology*

EFFECT OF VERATRINE AND EPINEPHRINE ON POLARIZED AND DEPOLARIZED ARTERY STRIP. Donald C. Brodie and Charlotte Schmid (intr. by H. H. Anderson.) School of Pharmacy and Dept. of Pharmacology, Univ. of Calif., San Francisco, Calif.

The effect of veratrine on the response of epinephrine in both polarized and depolarized strips of rabbit aorta was studied. Strips of aorta were mounted in chambers of Krebs solution containing calcium EDTA (10^{-5} g./ml.) Isotonic contractions were recorded on smoked paper. In bath concentrations not exceeding 10^{-6}, veratrine produced no response, whereas in concentrations of 10^{-5} and above marked contraction of the muscle occurred. Veratrine in concentrations of 10^{-6} produced an enhancement of the response to stimulation by epinephrine. Depolarization of the muscle with potassium-Ringer solution produced an immediate shortening of the muscle which was subsequently relaxed with glyceryl trinitrate and papaverine hydrochloride. In the relaxed depolarized state, epinephrine produced shortening of the muscle, whereas veratrine in maximal concentrations caused no contraction nor was there any enhancement of the response to stimulation by epinephrine. The conclusions are: (1) epinephrine can activate the contractile elements directly without mediation of membrane depolarization, (2) polarization of the membrane is essential for veratrine-induced contraction. Supported by PHS Grant H-4042 NIH.

4 *Pharmacology*

MODIFICATION OF THE PRESSOR EFFECTS OF TYRAMINE AND TRYPTAMINE BY IPRONIAZID AND RESERPINE. H.Burford* and Edward J. Walaszek Dept. of Pharmacology, Kansas Univ. Med. School, K.C., Kansas.

The pressor response of anesthetized dogs to tyramine and tryptamine are markedly potentiated by prior treatment with iproniazid (20 mg/kg) followed in 3 hours by reserpine (1 mg/kg). Studies with dogs receiving either iproniazid or reserpine reveal small increases in the pressor responses to the two amines, however the potentiation observed when both drugs are present is of such magnitude that it cannot be explained as pure addition. Experiments with adrenergic blocking agents, ganglionic blocking agents and ganglionic stimulants, lead to the conclusion that the potentiation of tyramine and tryptamine by the combination of iproniazid and reserpine can best be explained by the ability of these two amines to produce their pressor effects by displacing norepinephrine from vessel walls. This has been demonstrated for tyramine by Burn and Rand (J. Physiol. 144: 314, 1958). The effect of iproniazid seems to be related to monoamine oxidase inhibition while that of reserpine to a facilitation of the release of norepinephrine from the vessel walls by the two amines. These experiments suggested that the pressor effect of tyramine is mediated through norepinephrine release while that of tryptamine is mediated partly through the above mechanism and partly through a direct action of its own.

1 *Physiology*

ON THE PRESENCE OF A PERMEABILITY AND CHEMOTACTIC FACTOR IN BLOOD SERUM. Valy Menkin. Henry Phipps Institute, University of Pennsylvania, Philadelphia, Pa.

There is a factor in canine blood serum capable of increasing capillary permeability. This is demonstrable by the trypan blue test in rabbits. The factor is obtained by dialyzing blood serum at about 10°C. for 3-7 days against twice its volume of distilled water. The indiffusible fraction is adjusted to pH 4-4.5 with N acetic acid. A precipitate forms. This is separated by centrifugation and largely redissolved at about pH 7.0 with NaOH. The factor is active in inducing in the treated cutaneous area a homogenous accumulation of trypan blue from the blood similar to leukotaxine. The factor also causes emigration of polymorphonuclear leukocytes. Unlike leukotaxine it is non-diffusible and heat labile. It is inactivated by ACTH and cortisone. It fails to be inactivated by ribonuclease or peptidases. The permeability factor of blood serum is inactivated by chymotrypsin. DU spectrophotometric measurements indicate a peak rising from 265 mμ to 278-280 mμ. The available data are consistent with its being a nucleo-protein. Studies are in progress to characterize it further, and to determine its relation to the permeability PF/dil factor of serum described by Miles and his collaborators. Finally, this factor conceivably reinforces the effectiveness of leukotaxine in inflammation by seeping into the inflamed area.

2 *Physiology*

EVIDENCE FOR A BLOOD-BORNE VASODILATOR RELEASED FROM AN ISCHEMIC EXTREMITY. Chester Hyman and Bernard R. Freeburg* Dept. of Physiol., Univ. So. Calif. Sch. of Med., Los Angeles

The hypothesis that ischemia results in the formation of vasodilator substances was explored using plethysmographic measurements of forearm blood flow before, during and after arterial occlusion of a leg. In 12 subjects, the forearm blood flow typically decreased immediately after release of a 10 min. occlusion; a period of moderate hyperemia follows. The initial vasoconstriction might result directly or via reflexes from trapping of blood in the previously occluded leg. The role of this factor was studied in experiments where the leg was packed with blood by stepwise venous occlusion before the application of the final supersystolic occlusion. Here, although release of the leg cuff leads to minimum blood loss, the phase of arterial constriction in the forearm was only somewhat shorter but the dilatation was heightened. Experiments were performed in which mild exercise of the occluded limb increased the quantity of vasodilator material, and forearm blood flow after the release of the leg cuff. These increases in forearm flow and decreases in calculated arterial resistance after release of leg occlusion are consistent with the hypothesis of a circulating dilator agent produced in the ischemic limb. The mechanisms responsible for the initial vasoconstriction are not clear. (Supported by a PHS grant H-352 from the NHI)

3 *Pharmacology*

BLOCKADE OF THE BETA ADRENERGIC RECEPTOR. B. Levy* and R. P. Ahlquist. Dept. Pharmacology, Med. Coll. of Ga., Augusta, Ga.

The intravenous injection of ethylnorepinephrine into the anesthetized dog produces a transient pressor response followed by a more prolonged depressor response. Pretreatment with B-hydroxy-N-isopropyl-3,4 dichlorophenethylamine (DCI), a drug that selectively blocks the beta receptor, unmasks the potent vasoconstrictor effect of ethylnorepinephrine which now becomes a purely pressor drug. The present paper explores the possibility of using such a response as a test for blockade of the beta adrenergic receptor. If ethylnorepinephrine is given in a dose of 50 ug/kg, repeatedly, the response is always the same, namely: a transient pressor response followed by a more prolonged depressor response. Ethylnorepinephrine "reversal" will also occur after pretreatment with 2.5 mg/kg of phenylephrine. DCI reduces the depressor response to isoproterenol but it will not convert isoproterenol to a pressor drug. Therefore if pretreatment with a given drug results in both "reversal" of ethylnorepinephrine and reduction of the depressor response to isoproterenol without "reversal", then this is good evidence that the drug in question is acting through beta adrenergic blockade. Isoprophenamine (1-o-chlorophenyl-2-isopropylaminoethanol) has been found to be an effective, short acting beta adrenergic blocking agent. (Supported by grant H-3514 from NHI, USPHS.)

4 *Pharmacology*

STUDIES ON ISOXSUPRINE METABOLISM. John A. LaBudde (intr. by J. H. Weikel), Department of Pharmacology, Mead Johnson and Company, Evansville, Indiana.

After oral administration of isoxsuprine, 2-(phenoxy-2-propylamino)-1-(p-hydroxyphenyl)-1-propanol HCl, to rats, it was previously shown (Weikel et al., Tox. and Applied Pharm., 1: 579, 1959) that about 3% of the dose is excreted in the urine unchanged and 2/5 is excreted as an acid labile conjugate of isoxsuprine. This conjugate has been isolated by chromatography on ion-exchange resins and identified as a glucuronide of isoxsuprine. Paper chromatography of ether extracts of urine from rats given 600 mg/Kgm of isoxsuprine revealed the presence of three compounds in addition to isoxsuprine which were not present in control urine. These compounds, excreted in both free and conjugated forms, were isolated by chromatography on cellulose columns. On the basis of physical properties and color reactions one of these compounds appears to be a non-phenolic amine with basically the same structure as isoxsuprine. The other two compounds have been identified as phenolic amines. Results in various in vitro test systems indicate that these metabolites retain some of the smooth muscle inhibitory activity of the parent compound. The nature of this activity as well as the further chemical characterization of these compounds will be discussed.

075. CARDIOVASCULAR SYSTEM
Experimental CV Disease

1 *Physiology*

PULMONARY EMBOLISM IN SYMPATHECTOMIZED DOGS. S.A. Kabins and J. Fridman (intro. by R.Pick) Cardio. Dept., Med. Res. Inst., Michael Reese Hosp. and Med. Center, Chicago, Ill.

A localized pulmonary infarct was produced by the injection of a suspension of starch through a catheter into the pulmonary artery wedge position of one lung lobe in anesthetized dogs. Control and sham operated dogs uniformly showed 3-4 plus bilateral pulmonary edema and a rise in the pulmonary artery pressure. In sympathectomized dogs, edema was inhibited in the contralateral lobes and there was no appreciable rise in the pulmonary artery pressure. It is concluded that reflexes mediated through the sympathetic nervous system play a major role in the formation of pulmonary edema and pulmonary arteriolar constriction under these experimental conditions. (Supported by a grant from the United States Public Health Service, HTS-5252).

2 *Pharmacology*

ROLE OF ADRENAL CORTICOIDS IN RENIPRIVAL CARDIOVASCULAR NECROSIS. David Lehr and Marilyn Krukowski* Dept. of Physiol. and Pharmacol., New York Medical College, Flower and Fifth Avenue Hospitals, New York City.

It was demonstrated earlier that the parathyroid factor which induces cardiovascular necrosis in the rat following bilateral nephrectomy (Nx), was apparently released in sufficient excess only in the presence of adequate amounts of adrenal corticoids (Ann. New York Acad. Sci. 72: 901, 1959). This led to the search for a possible "trophic" effect of the adrenal cortex upon the parathyroid. Three groups of adrenalectomized rats, divided into appropriate subgroups, received either Cortisone, DCA or a combination of both, starting prior to Nx and continuing until death. The adrenals were removed either at the time of Nx (Group I) or 10 days prior to Nx (Groups II and III). Group II was maintained on saline, whereas Group III received adrenal corticoids instead. Under exposure to identical dosages of DCA or DCA-Cortisone, Group II exhibited a strikingly lower incidence of cardiovascular necrosis than Group I. Group III reacted like Group I. Thus, the absence of adrenal cortical hormones for at least 8 days prior to Nx, decreased the responsiveness of the parathyroid to these hormones. The findings are consistent with the concept of a trophic effect of the adrenal cortex upon the parathyroid. (Aided by grant No. H-890 [C9] from the National Heart Institute, PHS.)

3 *Pharmacology*

ON THE MECHANISM OF RENIPRIVAL CARDIOVASCULAR NECROSIS. Marilyn Krukowski* and David Lehr. Dept. of Physiol. and Pharmacol., N.Y. Med. Coll., Flower-Fifth Ave. Hosps., N.Y.C.

It was shown earlier that the appearance of severe cardiovascular and smooth muscle necrosis in reniprival rats was due to adrenal cortex mediated hyperparathyroidism. (Ann. New York Acad. Sci. 72: 901, 1959). The extent of involvement of mineral metabolic derangements in the ultimate mechanism of muscular necrosis was explored by serial serum electrolyte determinations in intact and parathyroidectomized (Px) rats following bilateral nephrectomy (Nx). In both groups inorganic phosphate (P) levels reached twice pre-Nx values in 24 hours and climbed to 3 and 4 times normal thereafter. Simultaneously, intact rats manifested pronounced hypercalcemia, whereas Px rats either maintained their pre-Nx hypocalcemia or showed a substantial further decline of calcium levels. In addition, all rats developed increasingly severe hyperkalemia. While muscular necrosis did not occur in the presence of pronounced hypocalcemia, no clear cut correlation was found between the extent of hypercalcemia or the size of the Ca/P product and the incidence and severity of lesions. Without ruling out the participation of Ca/P oversaturation in the mechanism of necrosis, the existence of a necrotizing factor is postulated. (Aided by grant No. H-890 (C9) from the Natl. Heart Inst., PHS)

4 *Pathology*

CARDIAC VALVULAR CHANGES IN HYPOPHYSECTOMIZED RATS COMPARED TO THE INTACT RAT.* Angrist, A., Oka, M. & Nakao, K. Albert Einstein Med. Coll., N.Y. 61, N.Y.

By previous work (Amer. Jour. Path. in press) hypertrophy of the adrenals was correlated with valvular lesions of rats after stress or hormone excess (DOCA, ACTH, etc.). Analysis of the role of hormones of adrenals in relation to those of the pituitary on the cardiac valve in the production of the lesions was attempted by the study of three groups of rats under the same conditions of stress: 1) intact 2) adrenalectomized and 3) hypophysectomized rats. In this study, hypophysectomized rats (129) were divided into groups as follows: 1) no treatment 2) cold 3) adrenalectomy 4) ACTH 5) DOCA 6) ACTH and DOCA in combination. In general, hypophysectomized rats showed less marked valvular changes than intact or adrenalectomized rats for same conditions and stresses. No marked changes were found in hypophysectomized animals with the multiple variables noted, with the exception of the combination of ACTH and DOCA which showed more marked edema and cellularity, and collagen changes and fibrinoid in some.

* Supported by USPHS Grants H-2190 (C2-3)

5 *Pathology*

MYOCARDIAL NECROSIS INDUCED BY TEMPORARY OCCLUSION OF A CORONARY ARTERY. R.B. Jennings, H.M. Sommers*, G.A. Smyth*, H.A. Flack*, and H. Linn*. Northwestern University Med. School, Chicago, Illinois

The circumflex branch of the left coronary artery of 115 dogs was temporarily occluded 0.5 to 2 cm. from its origin in order to determine how many minutes of ischemia are required to induce irreversible injury in dog myocardium. Occlusions of 5 to 60 minutes duration were used. After the desired time interval had elasped the coronary arterial circulation was restored. Dogs surviving release were killed 1-7 days later and studied to see if any necrosis was present in the myocardium supplied by this artery. 70 dogs died of ventricular fibrillation occurring simultaneously with the restoration of the circulation and were lost to the experiment. The results in 36 dogs that survived the occlusion without developing a thrombus in the artery are reported. No necrosis was noted in the affected myocardium in any dog receiving up to 18 minutes of ischemia. Subendocardial foci of necrosis appeared at 22 minutes in one dog and in all dogs given 25 or more minutes of ischemia. The amount of necrotic tissue in the portion of myocardium supplied by the circumflex artery increased with time, until at 60 minutes, the infarcts were of roughly the same size and distribution as those resulting from permanent occlusion of this vessel.

XUM

6 *Pathology*

THE DEVELOPMENT OF TISSUE DAMAGE IN COLD-INDUCED ISCHEMIA
J. Peter Kulka, M. D. Robert B. Brigham Hospital, Peter Bent Brigham Hospital, and Harvard Medical School

Cold-induced ischemia is important in the pathogenesis of frostbite, trench foot, and chilblains. Moreover, a similar mechanism of tissue damage might be concerned in other disease conditions which are associated with prolonged localized vasoconstriction. We observed the development of circulatory impairment in cold injury of the ears of mice kept individually caged at 3°C. A recently devised technique of sudden in situ freezing of the ears and dehydration in the cold permitted the correlation of both functional and organic vascular changes as seen in cleared whole mounts with histologic changes demonstrated in subsequently prepared tissue sections. Circulating plasma was visualized by parenteral injection of Evans Blue before fixation. In this manner, the following sequence of local circulatory changes was shown to precede the onset of gangrene: 1) cutaneous vasoconstriction with continued filling of muscle capillaries, 2) progressive segmental venular and capillary stasis associated with erythrocyte extravasation, 3) arteriolar-venular shunting, and 4) massive thrombosis. The development of cutaneous necrosis conformed to regions of confluent stasis. Thus it appears that loss of endothelial integrity in venules and capillaries with blocking of venous return by compaction of erythrocytes is the primary disorder leading to tissue damage.

076. CELLS

1 *Physiology*

FURTHER STUDIES ON MONOSACCHARIDE PENETRATION INTO HUMAN RED CELLS. R. G. Faust*[1] and A. K. Parpart. Dept. of Biology, Princeton University, Princeton, N.J.

The rate of penetration at 37°C of completely mutarotated D-glucose into human red cells has been studied at pH 6, 7 and 8 in relation to variations in the sugar concentration. Glucose penetrates more rapidly at pH 7 than at either pH 6 or 8 when the sugar concentration is greater than 0.2M. However, the rate of glucose penetration is identical at pH 6, 7 and 8 when the sugar concentration is less than 0.1M. Also a comparison of the rates of penetration of 0.3M α and β glucose has revealed that β glucose penetrates 4 times faster at pH 7 and 8 than α glucose. At pH 6 α and β glucose penetrate at the same rate. An altered diffusion mechanism for monosaccharide penetration into the human red cell has been postulated from these and other data previously reported.

[1](Supported by a Fellowship of the National Institutes of Health).

2 *Physiology*

ISOLATION AND PROPERTIES OF AMPHIBIAN EGG NUCLEOLI.[1] C.T. Crouse and F.J. Finamore (intr. by F.R. Hunter.) So. Ill. Univ., Carbondale, Ill.

After removal of the yolk platelets and pigment from amphibian egg homogenates by differential centrifugation, nucleoli can be precipitated from the supernatant fractions by the addition of Mg^{++}.

The isolated nucleoli have an average diameter of 6.1 microns and a specific gravity between 1.3 and 1.4.

Chemical analyses of these structures indicate that they contain no DNA, a negligible quantity of lipids, from 2-9% RNA, and about 95% protein.

These results indicate that amphibian egg nucleoli are chemically and physically similar to nucleoli of other species.

[1]Supported by USPH Grant No. 5994.

3 *Biochemistry*

SUBCELLULAR DISTRIBUTION OF SUBSTANCES IN SEEDS OF Arachis hypogaea. Julius W. Dieckert and James E. Snowden, Jr. (intr. by A. M. Altschul). SURDD, USDA, New Orleans, La.

The quiescent state in seeds is not well understood. One facet of the problem is the biochemical cytology of late maturation, quiescent and early germination stages of the seed. To learn more about this aspect of the problem, a study was made of the intracellular distribution of substances in the cotyledons of peanuts. Several subcellular components were isolated by homogenization and differential centrifugation in nonaqueous media. The most homogeneous fractions were: aleurone grains, protein bodies, starch grains, cell walls and a reticulum. Nuclei were not obtained pure. Analysis indicated that the aleurone grains are high in protein, phytin and ash. The protein bodies are high in protein and low in phytin. Starch grains are low in protein and phytin but high in starch. DNA followed nuclei only. The reticulum is high in protein, phosphatides and RNA. The presence of RNA here suggests the presence of important enzyme-forming elements that will become functional during germination. The cell wall fraction showed more nitrogen than expected on the basis of observed contamination by high protein particulates. This suggests that nonparticulate cytoplasmic material remains with the cell wall. These data confirm that a high degree of compartmentation exists in the quiescent cell.

4 *Pharmacology*

METABOLISM AND CILIO-ACCELERATOR ACTION OF 5-HYDROXYTRYPTOPHAN (5-HTP) IN GILL PLATES OF MYTILUS AND MODIOLUS. A.S. Milton* and R.E. Gosselin, Department of Pharmacology and Toxicology, Dartmouth Medical School, Hanover, New Hampshire.

Excised gill plates of Mytilus edulis and Modiolus demissus were superfused with sea water containing 5-HTP (5 to 100 μg/ml.), and ciliary activity was measured stroboscopically. The 5-HTP produced a large rise in the beat frequency of the lateral cilia. In contrast to the cilio-accelerator activity of 5-hydroxytryptamine (5-HT), the action of 5-HTP developed slowly and persisted for several hours. The gill plates of both species were found to contain small amounts of endogenous 5-HT as detected by photofluorometry and by bioassay. The amount increased several-fold when the intact isolated gill plate was incubated in sea water containing 5-HTP. Homogenates of Modiolus gill plates consumed oxygen in the presence of 5-HT and 5-HTP, presumably because of 5-hydroxyindole oxidase activity, which was first reported in the gill plate of Mytilus (Blaschko and Milton, J. Physiol. 148, 54P, 1959). These data give further support to the hypothesis that endogenous 5-HT is concerned in the physiological regulation of ciliary activity. (Supported by PHS research grant RG 5145).

5 *Nutrition*

NITROGEN AND PHOSPHORUS METABOLISM DURING THE SYNCHRONOUS GROWTH OF CHLORELLA PYRENOIDOSA. R. R. Schmidt (intr. by R. F. Miller.) Virginia Polytechnic Institute, Blacksburg, Va.

Cultures of the alga Chlorella have been synchronized and examined for shifts in the metabolism of the cells as they developed through a complete life cycle. Three methods were used in combination to synchronize the cells: (1) continuous illumination of low intensity, (2) intermittent illumination, and (3) fractional centrifugation. The synchronous growth of the cells was followed with measurements of total cell nitrogen, protein nitrogen, dry weight, nitrate and phosphate uptake, and cell number. A linear logarithmic increase in total cell nitrogen, protein nitrogen, and nitrate uptake was observed. Eighty to 90% of the phosphate was absorbed immediately prior to and for a short time during mitosis. Tentative evidence indicates that the phosphate accumulated in the cells as polyphosphate and was important for the division process. When the air stream coming from the culture was bubbled through a trap containing acid for a single synchronized culture cycle, the trap was found to contain organic nitrogen. White, needle-like crystals were precipitated from the acid solution by ether.

077. CELLS–Active Transport

1 **Physiology**

ACTIVE CHLORIDE TRANSPORT DURING INTESTINAL SECRETION. C.S. Tidball (introduced by C. A. M. Hogben). Physiology Department, The George Washington University, School of Medicine, Washington, D.C.

When the small intestine is excited to secrete, there is a net movement of Cl^- from extracellular fluid to lumen. A change from absorption to secretion was achieved in the isolated canine jejunal loop in situ by either the constant intravenous infusion of bethanechol or the incorporation into the luminal solution of 10^{-2} M/L of 2,4-dinitrophenol or 2,4,5-trichlorophenol. The solution introduced into the lumen was isosmolar with extracellular fluid, but contained 50 meq./L more Cl^- than the extracellular fluid. During secretion small (2-10 mv) transmural electrical potentials were measured with the luminal surface being negative with reference to the peritoneal surface. Thus, Cl^- moves against both an adverse chemical and electrical potential gradient and is being actively transported. It is noteworthy that the active transport of Cl^- can proceed in spite of the presence of high concentrations of the substituted phenols which uncouple oxidative phosphorylation.

2 **Physiology**

EFFECT OF PROTAMINE AND STROPHANTHIN ON THE ABILITY OF MITOCHONDRIA TO CONCENTRATE POTASSIUM. Leonard Share. Dept. of Physiology, Western Reserve Univ., Cleveland, Ohio.

The cardiac glycosides and certain basic polypeptides, such as protamine, inhibit potassium transport in a variety of intact cells by, presumably, a direct action upon the transport system. A study has been made of the effects of strophanthin and protamine on the ability of rat liver, kidney and heart mitochondria to concentrate potassium. The mitochondria were incubated in a medium which contained the essentials for oxidative phosphorylation but without added potassium; potassium was added to the medium at the end of one hour. Both protamine and strophanthin impaired the ability of the mitochondria to concentrate potassium in this test system. These substances also induced swelling of the mitochondria, but had little or no effect on mitochondrial sodium concentration. Protamine increased oxygen consumption in liver mitochondria and diminished the stimulatory effect of 2,4-dinitrophenol, suggesting that oxidation was uncoupled from phosphorylation. The minimum effective concentrations of protamine and strophanthin were approximately 0.004% and 1% respectively. It is concluded that the actions of protamine and strophanthin on mitochondria are quantitatively and qualitatively different from the actions of these substances on intact cells. (Supported by a grant from the Public Health Service.)

3 **Physiology**

FROG SKIN EPITHELIAL CELL MEMBRANE RESISTANCE. T. Hoshiko. Dept. of Physiology, Western Reserve Univ. Cleveland, Ohio.

The d.c. resistance of the outermost potential barrier of isolated frog skin (R. pipiens) was calculated as a fraction of the total skin resistance. The isolated skin bathed in sulfate Ringer's was micropunctured from the physiological outside as described previously (Acta physiol. scand. 39:348, 1957). When the first stable potential jump was encountered (positive with respect to the outside bathing medium), d.c. current of increasing strength was passed through the skin via "short circuiting" current electrodes. Fine glass bridges positioned close to the inner and outer skin surfaces were used to record the total skin potential. The microelectrode potential was fed into the vertical amplifier of an oscilloscope and the total skin potential into the horizontal amplifier. The oscilloscope trace resulting from the passage of increasing current was a plot of the first potential jump as a function of the total skin potential. The slope of this line is equal to the resistance of the outer potential barrier expressed as a fraction of the total skin resistance. In all "good" punctures almost all the skin resistance was located across the outer potential barrier, indicating that the presumed sodium permeability of the outer border is much lower than the potassium permeability of the inner border of the skin epithelial cell.

4 **Physiology**

AMINO ACID ACTIVE TRANSPORT AND EXCHANGE DIFFUSION IN EHRLICH ASCITES CELLS. John A. Jacquez. Sloan-Kettering Inst. for Cancer Research, N. Y.

The initial flux of uptake of L-tryptophan has been measured in Ehrlich ascites cells preincubated in Krebs-Ringer solution (F_1) or in Krebs-Ringer containing the amino acid azaserine (F_2). The flux F_1 is primarily an active transport flux whereas F_2 contains both active transport and exchange diffusion components. Dinitrophenol and cyanide markedly inhibit F_1 and slightly inhibit F_2, giving a small increase in the flux difference F_2-F_1. These and other data have been compared with the predictions of a number of models of active transport systems. Two types of carrier models best fit the experimental data. These differ in the nature of the linkage between the carrier system and cellular metabolism. In the Carrier Active Transport model the carrier is actively transported from the inner to the outer surface of the cell membrane. In the Carrier-Substrate Active Transport model the dissociation of the carrier-substrate complex is accelerated at the inner surface of the cell membrane. Both models give the same conditions on the rate constants for the flux difference F_2-F_1 to be greater than zero. In the first model, active transport and exchange diffusion compete for the carrier; in the second model the reactions of active transport are included in the exchange diffusion loop.

5 **Physiology**

THE LINK BETWEEN METABOLISM AND THE ACTIVE TRANSPORT OF Na IN HUMAN RED CELL GHOSTS. Joseph F. Hoffman. National Heart Institute, Bethesda, Maryland.

The characteristics of the active transport of Na by ghosts has been previously described (Biophys. Soc. Abst.1960). This system is employed to assay metabolic intermediates for their role in driving the Na pump. Compounds normally non-penetrating are incorporated inside the ghost during hemolysis. The results show that ATP alone is the direct energizer of the pump and that any reaction that will generate ATP will run the pump. Thus, ADP or PEP stimulate the pump but are less effective than ATP. In reactions involving PEP or inosine, ADP as a phosphate acceptor has to be present in catalytic amounts to activate the pump. Inosine without ADP is metabolized to lactate and generates ITP. However, ITP will not stimulate Na outflux. Given these same conditions (no ADP) adenosine will stimulate the pump. When a trace of ADP is present both inosine and adenosine will stimulate the pump. Thus, there exists a pathway to make ATP and ITP from their nucleosides but the pump is specific for the purine base, adenine. Inhibitors such as iodoacetate and arsenate do not effect the pump when it is driven by the utilization of ATP. These results relate the in vitro observations of Post (Fed.Proc.18:121, 1959) to a functional process and support the concept that the pump complex is or has as an intimate component an ATPase.

6 **Physiology**

POTASSIUM FLUXES ACROSS THE MEMBRANE OF THE EHRLICH MOUSE ASCITES TUMOR CELL: EVIDENCE FOR AUTO-INHIBITION BY EXTERNAL POTASSIUM. H.G. Hempling, Dept. of Physiology, Cornell Univ. Medical College, N.Y., N.Y.

The effect of external K on K fluxes has been studied to define the several pathways by which K crosses the membrane of the tumor cell. The external concentration of K has been varied from 5 to 100 meqs/liter by the replacement of Na, and measurements of K efflux, influx, and cell K content have been made with isotopic and flame photometric methods. K efflux decreases with increasing external K and goes through a minimum at 18 to 20 meqs/liter, at which point efflux has decreased 50%. At concentrations greater than 20 meq/liter, K efflux increases with increase in external K. K influx follows a similar pattern. These latter observations were based both on direct measurement and the additional fact that cell K does not change to the extent predicted from an effect on efflux alone. Fluxes are unchanged when Na is replaced with choline instead of K, in the inhibitory range. The inhibitory effect is proportional to the external K between 5 meq/liter and 20 meq/liter. This inhibitory effect is similar to that observed in Sepia axons, although it has not been reported for the human red cell. On the other hand, increase of efflux with increase in external K is compatible with exchange diffusion. (Supported by USPHS grant C-3690)

7 *Physiology*

AN ENZYMATIC BASIS FOR THE DIFFERENCE IN ACTIVE CATION TRANSPORT IN TWO GENETIC TYPES OF SHEEP RED CELLS. D. C. Tosteson, R. H. Moulton* and M. Blaustein*. Dept. Physiol. Washington Univ. Schl. Med., St. Louis.

One reason for the difference in cation composition of sheep red cells with high intracellular K (HK) and low K (LK) is that the K-Na pump works 3-4 times faster in the former genetic type (Tosteson and Hoffman, Proc. Biophys. Soc., '59). ATP can serve as the sole source of energy for the K-Na pump in human red cells. An ATPase which requires K and Na for maximal activity has been isolated from crab nerve (Skou, Biochim. Biophys. Acta, 23:394, '57) and human red cells (Post, Fed. Proc. 18:121, '59). A white particulate enzyme preparation was made from washed cells lysed in KCl or NaCl (.17M) plus 0.1% digitonin. K-Na dependent ("pump") ATPase, measured as the extra inorganic P released from ATP by incubation in both K (.034M) and Na (.076M) above that released in either ion alone (.11M), was 3-4 times higher in HK than in LK cells. 5×10^{-5} M strophanthidin which blocks the K-Na pump also blocks "pump" ATPase. These results suggest that this ATPase is an integral part of the K-Na pump. (Supported by U. S. P. H. S. H-4045.)

8 *Physiology*

HIGH K AND LOW Na MOBILITY IN CELLS; ITS INTERPRETATION IN TERMS OF THE FIXED CHARGE-INDUCTION HYPOTHESIS. G. LING. EASTERN PENNA. PSYCHIATRIC INSTITUTE, PHILADELPHIA

According to the fixed charge hypothesis entry of cation, C_2^* into and its diffusion within an anionic fixed charge system involves association with a fixed anion (F^-) and its subsequent displacement by another cation, C_3; potential barriers prevent most cations to travel continually through interstices among fixed charge-counterion pairs (C_1F^-) (Amer. J. Phys. Med. 179:659, '55): (1) $C_1F^- + C_2^* \rightleftarrows F^-C_2^* + C_1 + \Delta H_1$; (2) $C_2^*F^- + C_3 \rightleftarrows C_3F^- + \frac{1}{2}C_2^*(in) + \frac{1}{2}C_2^*(out) + \Delta H_2$. It can be shown that for these adsorption reactions the activation energy, E, unlike ordinary chemical reactions, is virtually equal to the heat content change $\Delta H_1, \Delta H_2$. Thus if $C_2^* = K^{42}$, $C_1 = C_3 = K$, the overall E should be smaller than if $C_2^* = K^{42}$, $C_1 = K$ but $C_3 = Na$. These conditions obtain in studies of the initial rate of entry of K^{42} into frog muscles in KCl and in NaCl. Supporting the theory the E of K^{42} entry (0-30°C) was found to be 5.65 Kcal/M. in isotnic NaCl; only 2.58 in isotonic KCl (9 sets of expts.); it was 7.26 in 20 mM NaCl-glucose only 3.80 in 20 mM KCl-glucose (3 sets of expts.); Similarly K^{42} diffusion within nerve and muscle cells which involves a process in which $C_1 = C_2^* = C_3 = K^+$ should be fast as was shown by Hodgkin and Keynes (J. Physiol. 119: 513, '53); while Na^+ diffusion ($C_1 = C_3 = K^+$, but $C_2^* = Na$) should be slow as was also shown by Müller in nerve fibers (J. Expt. Cell. Res. Supp. 5:118, '58).

9 *Physiology*

THE EFFECTS OF CHLORPROMAZINE ON THE RED CELL. R. E. Eckel. Western Reserve University School of Medicine, Cleveland, Ohio.

Human defibrinated blood, cold stored 3-5 days, has been washed and incubated in a saline phosphate buffer at 37°C. in air or nitrogen with inosine as substrate. Addition of chlorpromazine (CZ) at a final concentration of 0.0005 M results in moderate immediate hemolysis and increased osmotic fragility (more in hypotonic K solutions than in hypotonic Na solutions). Both are progressive over a 3 hour incubation without change in cell volume. These cells fail to reaccumulate, or actually lose, K. K^{42} studies show that the inward rate of K movement (Mi) is unaffected. The outward rate (Mo) is accelerated. Mi approaches zero and Mo is slightly decreased in the presence of CZ and ouabain. Addition of K^{42} at various times after addition of CZ reveals no gross heterogeneity of cell K. K fluxes in cells incubated 3 hours in CZ, washed, and reincubated, revert towards normal. The membrane damage caused by CZ does not affect active K transport.

10 *Physiology*

IONIC EFFECT ON SUGAR TRANSPORT FROM THE INTESTINE OF THE RAT. T. Z. Csaky. University of North Carolina School of Medicine, Chapel Hill, N. C.

It was found previously that in the isolated intestine of the toad Na ions are essential on the mucosal surface for the functioning of the sugar pump (Csaky and Thale: J. Physiol. in press). In the course of the present investigation a loop of jejunum of the anesthetized rat was perfused with isosmotic solutions of either Na_2SO_4, Li_2SO_4, K_2SO_4, $MgSO_4$ or mannitol. 50 mg % glucose was dissolved in the perfusing fluid and the rate of glucose absorption was measured. The highest glucose influx was observed from Na_2SO_4 then from mannitol > $MgSO_4$ > Na_2SO_4 > Li_2SO_4. However the glucose influx increased in each experiment if the initial mannitol, $MgSO_4$, K_2SO_4 or Li_2SO_4 medium was replaced by Na_2SO_4 in the same loop. The results confirm those obtained in the toad intestine and suggest a possible link between sugar pump and sodium pump. (Supported by a grant from U. S. Public Health Service.)

11 *Physiology*

THE TRANSPORT OF MONOVALENT IONS ACROSS THE ISOLATED SMALL INTESTINE OF THE RAT. T. W. Clarkson* and A. Rothstein. Sch. of Med. and Dent., Univ. of Rochester, Rochester, New York.

The net transport of monovalent ions by everted segments of rat small intestine was measured chemically. Na^+ was always found to be transported against the total electrochemical gradient. Other cations, $(C_2H_5)_3NH^+$, Cs^+, K^+ and Li^+ and the anions, Cl^-, CNS^-, HCO_3^-, IO_3^- and NO_3^- consistently moved down the gradient. The mean flux ratio for Na^{22} (4 experiments) was $2.2 \pm .2$. The flux ratio as predicted from the electrochemical gradient was $0.5 \pm .2$. The found flux ratio for Cl^{36} of $2.4 \pm .8$ (4 experiments) was insignificantly different from the predicted value of $2.5 \pm .4$. The mucosal to serosal fluxes of Na^{22} and Cl^{36} between 2°C and 21°C remained constant at .12 μEq/mg. dry wt/hr. At higher temperatures, the fluxes increased rapidly until at 37°C, the Na^{22} flux was $1.8 \pm .6$ and Cl^{36} was $1.1 \pm .2$ μEq/mg. dry wt/hr. The significance of these results will be discussed in relation to the mechanism of transport of monovalent ions by the small intestine. (Work performed under contract with the U. S. Atomic Energy Commission at the University of Rochester Atomic Energy Project, Rochester, New York.)

12 *Physiology*

RELATIONSHIP BETWEEN ION TRANSPORT AND ELECTRICAL IMPEDANCE IN GASTRIC MUCOSA by Warren H. Dennis (intr. by Warren S. Rehm) Univ. of Louisville, Louisville, Kentucky

The impedance locus diagram of the non-acid secreting gastric mucosa is characterized by two arcs. The characteristic frequencies are 3-5 cps and 200-500 cps. When the mucosa is stimulated with subcutaneous histamine and mecholyl qualitative and quantitative impedance changes occur with the frequency range 0.5 to 50 cps the mucosa is purely resistive. However, the mucosa exhibits the unique property of a purely resistive impedance that decreases in magnitude with increasing frequency. The changes in the arc with the higher characteristic frequency are correlated with the rate of secretion. The characteristic frequency increases to about 5Kcps and the width of the arc decreases. In the resting mucosa the two reactive arcs correspond to capacitances of 1000 uf/cm^2 and 3-4 uf/cm^2. The secreting mucosa shows only the higher frequency arc and the capacitance decreases to 0.5-1 uf/cm^2. (This work supported by USPHS grant A-2882)

13 *Biochemistry*

THE "DOWNHILL" TRANSPORT OF AMINO ACIDS, FATTY ACIDS, AND SUGARS INTO BACTERIAL PROTOPLASTS AND THE ROLE OF GLYCOLYSIS AND K^+ IONS. A. Abrams, G. Yamasaki*, J. Redman*, and C. G. Mackenzie. Biochem. Dept., Univ. of Col. School of Med., Denver

The osmotic stabilization of S. fecalis protoplasts by oligosaccharides has shown that the cell membrane is impermeable to this class of compounds. (A. Abrams J.B.C. 234 383, 1959.) The permeability of a number of additional organic compounds has now been tested, including amino acids, fatty acids and monosaccharides. The L-amino acids with a molecular weight greater than serine (with the exception of valine) are osmotic stabilizers. However, D-alanine and D-serine are stabilizers in contrast to their L-analogues. The fatty acids, C_1 to C_5, the common dicarboxylic acids, and citrate are osmotic stabilizers. Monosaccharides are inactive as stabilizers. Upon the addition of glucose, protoplasts stabilized with amino acids or fatty acids undergo rapid metabolic swelling which may terminate in complete lysis. As is the case with oligosaccharides, K^+ is also required in the presence of the fatty acids, with the exception of acetate. The rate of metabolic swelling is inversely proportional to the number of carbon atoms in the fatty acid. These findings suggest that the cell membrane changes during glycolysis to permit the "downhill" penetration of diverse compounds which otherwise penetrate slowly if at all.

14 *Biochemistry*

THE UPTAKE OF ACETATE BY RAT DIAPHRAGM. Ernest C. Foulkes and C.M. Paine*, May Institute, Jewish Hospital and Dept. of Physiol., U. of Cincinnati, College of Medicine, Cincinnati,O.

The apparent discrepancy between the ready physiological diffusibility of such anions as acetate and lactate on the one hand, and on the other hand, the relative impermeability to anions of muscle tissue in vitro, led to an investigation of anion uptake by excised rat diaphragm. Measurements were carried out with the aid of C^{14}-labelled acetate over time periods not exceeding 25 seconds at 37°C. Under these conditions the tissue level of acetate remains well below that in the medium, and the leakage through the cut end of the fibres can be neglected. The uptake of acetate can then be described as the sum of two exponential terms, (A) and (B). Process (A) rapidly equilibrates with 18% of the tissue by volume; it is independent of acetate concentration up to levels as high as 50mM and can be observed even in the cold. Process (B) is absent at lower temperatures; it is saturated at relatively low levels of acetate in the medium (K_m about 2 mM) and can also be suppressed by other organic anions such as lactate. We identify process (A) with the diffusion of acetate into the extracellular fluid space of the tissue, and (B) with cellular uptake. Electroneutrality during anion uptake appears to be maintained by simultaneous cation movement, as process (B) is reversibly abolished in isotonic mannitol solution.

15 *Biochemistry*

EXCHANGE AND NET ACCUMULATION OF RADIOSULFATE BY MITOCHONDRIA. R.W. Winters,* A.M. Delluva,* J. Rosenbloom* and R.E. Davies. Physiology and Biochem. Depts., Univ. of Pennsylvania, Philadelphia.

The degree to which isotope exchange and net accumulation of sulfate contribute to the observed uptake of S^{35} by mitochondria of rat kidney cortex in media containing radiosulfate (as reported previously, Fed. Proc. 18, 353, 1959) has been studied. The "residual" sulfate of twice washed mitochondria, estimated by an indirect isotope dilution method involving labelling in vivo, was found to be approximately 430 µM in mitochondrial water. Under the in vitro conditions used to demonstrate accumulation of S^{35}, only about 0.5% (or 2.1 µM) of this residual sulfate was rapidly exchangeable. Since observed isotopic gradients between mitochondrial water and medium always considerably exceed those predicted from this exchange alone when the external K_2SO_4 concentration exceeds 1 µM, it is concluded that net accumulation of sulfate from the medium is the major process in the accumulation of the in vitro isotope. The newly accumulated sulfate itself was found to be rapidly and virtually completely exchangeable. The pH optimum for uptake in the absence of buffers was about 5.5. (Supported by USPHS Grant RG-6444(A).)

16 *Biochemistry*

GLUCOSE UTILIZATION IN A MUTANT STRAIN OF E. COLI. C. Asensio,* G. Avigad* and B. L. Horecker. N.Y.U. Col. of Med., New York.

The E. coli mutant MM6 (Monod) fails to grow when transferred from nutrient broth to synthetic glucose medium. Galactose, lactose or L-arabinose will support growth but not maltose, mannose, fructose, acetate, succinate or glucose. However, cells obtained by growth on broth or galactose will oxidize glucose. In the presence of galactose glucose is utilized for growth, the former preferentially. During growth on lactose glucose is excreted until all the lactose has been utilized. Cells from nutrient broth have normal glucokinase, galactokinase and phosphoglucomutase, but are devoid of UDPG-pyrophosphorylase, which appears during growth on galactose. The permease for α-methylglucoside shows full activity only after growth on glucose. Marked changes in cell morphology and motility accompany growth. It is suggested that the observed differences are related to the production of cell walls having differing composition and structure. (Supported by a grant from the National Institutes of Health).

17 *Biochemistry*

RATE OF GROWTH HORMONE STIMULATION OF AMINO ACID UPTAKE BY RAT TISSUES IN VIVO. Thomas R. Riggs and Lois M. Walker.* Dept. of Biol. Chem., The Univ. of Michigan, Ann Arbor.

Experiments were made to find out if a primary action of growth hormone on protein metabolism is to intensify transfer of free amino acids into cells. Male hypophysectomized rats (80-90 gms.) were injected with the non-metabolizable amino acid, C^{14}-α-aminoisobutyric acid (AIB). Animals were sacrificed 24 hours later and serum and tissue extracts counted to determine the distribution of the amino acid. Bovine growth hormone (Armour), 1 mg/rat intraperitoneally, caused a drop in serum AIB in 15 minutes, with a minimum by 1 hour (about 2/3 normal). The total AIB lost from the serum was too small to be detected as increases in any of the 5 tissues studied. The increased degree of concentration was evident, however, taking into account the following result: Mice whose extracellular AIB was diluted by large volumes of ascitic fluid showed that tissues lose AIB readily when serum levels are brought down. The failure of tissue AIB levels of growth hormone-treated rats to drop in response to the serum decreases therefore can be taken to indicate an increased ability of their tissues to concentrate the amino acid. Time studies will also be reported showing the rate of AIB uptake by rat tissues in the presence and absence of injected growth hormone.

18 *Biochemistry*

AMINO ACID CONCENTRATION STIMULATED BY ESTROGEN SULFATES. Halvor N. Christensen, Thomas R. Riggs and Judith C. Jones.* Dept. of Biol. Chem., The Univ. of Michigan, Ann Arbor.

The disulfates of β-estradiol and stilbestrol at levels above 5×10^{-4} M strongly stimulate the concentration of glycine and α-aminoisobutyric acid into Ehrlich ascites tumor cells, from synthetic media although not from the protein-containing ascitic plasma. At high glycine levels the extra glycine taken up greatly exceeds the molar equivalents of the estrogen added. We tentatively postulate that three groups of anionic agents (estrogen sulfates, auxins [J. Biol. Chem. 209, 413 (1954)] and pyridoxal phosphate) all fix at a common cationic site on the plasma membrane, a site that ordinarily serves to restrain a type of K^+ escape which drives amino acids into the cell (J. Biol. Chem. 222, 1479 [1958]). None of the three types enters the cell readily; their effects are not additive and are readily reversed by dilution; all are fixed on the cell by sites too numerous to be easily saturated. The simultaneous presence of estradiolsulfate decreases pyridoxal phosphate fixation on the cell, also on certain apoenzymes (Mason and Gullekson, J. Am. Chem. Soc. 81, 1517 [1959]) and at a specific site on the bovine serum albumin molecule. Competition for binding among these 3 types of substances and the basis of their action is still under study. (Supported in part by a grant from the National Cancer Institute, U.S.P.H.S.)

XUM

19 *Biochemistry*

DEPENDENCE OF AMINO ACID UPTAKE IN *L. ARABINOSUS* ON OSMOTIC FACTORS. Joseph T. Holden. Dept. Biochem., Med. Res. Inst., City of Hope Med. Center, Duarte, Calif.

A vitamin B_6 deficiency in *L. arabinosus* markedly reduces the amount of glutamic acid which can be taken up from buffers and retained intracellularly (*J. Biol. Chem.* 234, 865, 872 (1959)). Accumulation of alanine and proline are affected similarly. With B_6-deficient cells high concentrations of sucrose, galactose, lysine, KCl, KBr and NH_4Cl increase the energy-dependent uptake of these amino acids to maximal levels. Glycerol and ribose do not stimulate accumulation. Sucrose and KCl show exactly equal stimulations at a number of osmolal concentrations. Sucrose supplementation also corrects abnormal amino acid uptake observed with various cell types in which cell wall formation is incomplete. Evidence will be presented showing that the accumulated amino acids increase osmotic lability of B_6-deficient cells and that cessation of accumulation at low levels in these cells is not caused by a permanent inactivation of the uptake mechanism. These findings indicate that amino acids accumulated within *L. arabinosus* are osmotically active. They also support previous suggestions that amino acid uptake in B_6-deficient cells of this organism is limited by cell wall changes which reduce its ability to restrain intracellular osmotic forces.

20 *Biochemistry*

A COMMON PATHWAY FOR SUGAR TRANSPORT BY THE INTESTINE. Charles R. Jorgensen*, Bernard R. Landau* and T. Hastings Wilson. Depts. of Physiology and Biological Chemistry, Harvard Medical School, Boston, Massachusetts.

The competition between different sugars for the transport mechanism of hamster small intestine was tested with the *in vitro* method of Crane and Wilson (*J. Appl. Physiol.* 12:145, 1958). With this method a single segment of intestine may be used for both control and experimental periods. The transport of galactose (5mM) was inhibited by other sugars known to be actively absorbed by the intestine; namely, glucose (5mM), α-methylglucoside (5mM), 1-deoxyglucose (5mM), 6-deoxyglucose (5mM) and 3-O-methylglucose (25mM). On the other hand mannose and xylose, two sugars not actively absorbed, do not inhibit galactose absorption. The transport of 6-deoxyglucose (1mM) was inhibited by both 1-deoxyglucose (10mM) and glucose (12mM). The movement of the test sugar was always against a concentration gradient. In all experiments with inhibitors the rate of transport of the test sugar (galactose or 6-deoxyglucose) increased considerably when the tissue was removed from the solution containing both inhibitor and substrate and placed in a solution containing only substrate, indicating a displacement of inhibitor by test substrate. These experiments are consistent with the view that all transported sugars tested compete for a common pathway in hamster intestine.

21 *Biochemistry*

PHOSPHATIDIC ACID AS A CARRIER FOR Na^+ IONS. Lowell E. Hokin and Mabel R. Hokin. Department of Physiological Chemistry, University of Wisconsin, Madison, Wis.

The following data give further support to the hypothesis (Nature, 184:1068, 1959) that phosphatidic acid participates in a cyclic carrier mechanism for the transport of Na^+ ions across the apical membranes of the avian salt gland. After differential centrifugation of homogenates of the tissue, the enzymes diglyceride kinase and phosphatidic acid phosphatase, which catalyze the cycle, were found in high concentrations in the membrane fraction of the salt gland cell. Differential centrifugation after incubation of the tissue with P^{32} showed that the acetylcholine-stimulated turnover of phosphatidic acid, which reflects increased activity of the cycle, was localized in the membrane fraction. The portion of the phosphatidic acid of the cell which is involved in the increased turnover reached isotopic equilibrium within 15 minutes. At this time the net specific activity of the total phosphatidic acid was higher than that of any of the other phosphatides measured. When Na^+ ions were omitted from the medium and replaced with either sucrose or K^+ ions, there was no increased turnover of phosphatidic acid in the presence of acetylcholine. The studies indicated that phosphoinositide may also be a carrier for Na^+ ions.

22 *Biochemistry*

STUDIES OF THE RELEASE OF AMINO ACIDS FROM THE BRAIN. Abel Lajtha and Jeno Toth.* N. Y. State Psychiatric Inst. and Columbia University, New York.

In an investigation of the mechanism of amino acid entrance into and exit from the brain, the fate of intracerebrally administered C^{14} amino acids was studied. After the intracisternal injection the amino acids spread very rapidly and within minutes the amino acid was distributed fairly equally in the six parts into which the brain was sectioned. Subsequently the concentration of the administered amino acid decreased, that of l- and d-lysine slowly (with two-thirds of the dose still present after two hours), and that of leucine, phenylalanine, and proline rapidly. Glutamic acid and glutamine decreased with intermediate speeds between lysine and leucine. The specific activities of the amino acids decreased faster than their concentration; such changes are dependent on the metabolism of amino acids and proteins as well as, to a major degree, on the rate of exchange between blood and brain amino acids. This rapid exchange found previously in our laboratory thus occurs in spite of a several-fold initial increase in brain amino acid concentrations. At present there is no direct relation seen between the rate of uptake and release of single amino acids by the brain. The influence of the experimental conditions on the physiological mechanisms is a subject of further investigation.

23 *Biochemistry*

UPTAKE OF AMINO ACIDS BY "GHOSTS" OF MUTANT STRAINS OF E.COLI. H. Ronald Kaback (intr. by J. Wittenburg) Albert Einstein College of Medicine, N. Y.

Bacterial cytoplasmic membranes, "ghosts," prepared by distilled water lysis of "spheroplasts" of E. coli, are shown to retain the ability characteristic of the whole cells to take up amino acids. The WS mutant takes up roughly 80% as much glycine as the wild type; whereas "ghosts" of the WS mutant take up roughly 11% as much glycine as "ghosts" of the wild type. The WC mutant takes up roughly 26% as much lysine as thewild type; whereas "ghosts" of the WC mutant take up roughly 15% as much lysine as "ghosts" of the wild type. "Ghosts" of both mutants, when incubated with an "uninvolved" amino acid--leucine--take up as much as "ghosts" of the wild type. It may be concluded that at least some of the specificity of thewhole cells for the uptake of amino acids resides in the "ghosts;" and the "ghosts" can accumulate amino acids.

24 *Pharmacology*

Na^{24} AND K^{42} EXCHANGE IN ISOLATED CHICK EMBRYO HEART. R. L. Klein (intr. by W. C. Holland), Dept. of Pharmacology, Univ. of Miss. Medical Center, Jackson, Mississippi.

An investigation of the nature of unidirectional fluxes of Na and K has been undertaken using isolated chick embryo hearts. Intracellular K (70 mM) is 90-100% exchangeable using 6-8 day old ventricles in oxygenated Ringer's medium at 30°C. K influx is 0.019 and efflux 0.020μ moles/sec/gm wet tissue. The half life for efflux is 41 minutes. Between 20° and 30°, the Q_{10} for influx is 3.6 and for efflux 1.6, which suggests an early dependence of the embryo heart on an 'active' mechanism for K entry. Although the ventricular tissue contains similar levels of Na and K at 7 days, less than 10% of the total Na appears to be exchangeable. At 30° influx is 0.008 and efflux 0.09μ moles/sec/gm wet tissue. The half life for efflux is 9 minutes. Q_{10}'s for Na are 1.6 for influx and 1.3 for efflux. Preliminary results suggest that ventricular cells are relatively impermeable to Na and that a metabolically dependent Na extrusion mechanism is absent at 7 days.

25 *Nutrition*

EFFECT OF RADIATION ON PLACENTAL TRANSFER OF P-32. J. P. Feaster* and G. K. Davis. Nutrition Laboratory, Univ. Fla., Gainesville.

Radiation is known to increase capillary permeability. To determine effects on the placenta, pregnant rats received 200r whole-body Co-60 radiation (single dose); 48 hours later P-32 was given i.m. and the rats sacrificed 48 hours after dosing with P-32. Non-irradiated pregnant rats sacrificed 48 hours after i.m. administration of P-32 served as controls. In both irradiated and control groups there was a rather sharp increase in the amount of P-32 present per fetus with each added day of age, but the increase exceeded rate of fetal growth only slightly (i.e. increase in P-32 per gram of fetal tissue was small). More P-32 was found in fetuses from irradiated rats than in controls at all stages studied (16-22days), the difference being significant in fetuses of 16, 18, 19, 21 and 22 days (irradiated at 12, 14, 15, 17 and 18 days), indicating increased placental membrane permeability. Maternal tissue P-32 and total P levels were not affected by radiation. Urinary P-32 excretion was significantly higher, fecal P-32 slightly lower, in irradiated females.

26 *Immunology*

TRANSPORT OF GALACTOSE IN E. COLI. M. J. Osborn and W. L. McLellan, Jr. (intr. by A. W. Bernheimer). N. Y. U. Col. of Med., New York.

Transport of galactose into the cell can be studied independently of galactose metabolism in the galactokinase-less mutant of E. coli, ML32-400(Monod). Accumulated galactose-C^{14} is not readily washed out, but is quantitatively displaced by galactose-C^{12} or β-methylgalactoside, also a substrate for galactose permease. Galactose accumulation is sensitive to dinitrophenol; in contrast, accumulation of α-methylglucoside is unaffected by DNP. Inhibition of permease activity by DNP cannot be ascribed solely to an effect on the entry process. Concentrations of DNP which substantially depress the steady state level of internal galactose have little effect on its initial rate of entry. In the parent strain, ML30, oxygen consumption from galactose is stimulated by levels of DNP which inhibit galactose accumulation in ML32-400. Similarly, thiodigalactoside accumulation, but not hydrolysis of o-nitrophenylgalactoside, is inhibited by DNP in the constitutive lac^+ strain, ML308. DNP appears to inhibit intracellular accumulation of the substrate, rather than transport, per se. (Supported by a grant from the National Institutes of Health).

078. CELLS–Cytochemistry

Physiology

ADENYLATE KINASE OF NUCLEI FROM NORMAL AND NEOPLASTIC TISSUES. Leo A. Miller* and Anna Goldfeder. Cancer Research Laboratory, Dept. of Hospitals and New York University.

The presence of adenylate kinase in cell nuclei has not been heretofore demonstrated. Mitochondrial and microsomal sites for this enzyme, however, have been established. To show that this enzyme is also associated with the nuclear fraction, nuclei from mouse mammary tumors (spontaneous and transplanted) and from liver, all indigenous to the same inbred hosts, were isolated by a modification of the Philpot-Stanier method and by the Chauveau method. ATPase was found in the nuclei fractions and tended to mask the presence of adenylate kinase. Lysis of the nuclei with water and centrifugation at 100,000 x g(average) for 60 minutes separated the ATPase activity associated with the nuclear particulate material from adenylate kinase in the soluble fraction. Adenylate kinase was found in the soluble portion of the nuclei from the aforementioned tissues. The activity was lower in nuclei isolated by the Chauveau method. The nuclear fraction of the neoplastic tissues contained approximately 60% of the total activity of the combined fractions (nuclear and cytoplasmic) of the whole homogenate. The highest enzymatic activity was found in the nuclear soluble portion. Some activity was associated with the nuclear particulate material and could not be mechanically removed. (Supported by USPHS Grant C-2565(C4)).

2 *Physiology*

THE DIALYZABLE CONSTITUENTS OF SQUID NERVE AXOPLASM. Gottfried G. J. Deffner and Reimer E. Hafter (intr. by F. O. Schmitt.) Dept. of Biology, M. I. T., Cambridge, Mass.

A combination of preparative paper electrophoresis and paper chromatography has been used for analysis of the dialyzable constituents of axoplasm obtained from Loligo pealii and Dosidicus gigas. At present, about 95% of these constituents have been identified and quantitatively estimated. The same components have been found in the axoplasm of both types of squid, but frequently in very different proportions. A variety of amino acids, betaines, poly-functional alcohols and carbohydrates were detected in this material for the first time. Isethionic acid, taurine, aspartic acid, glycocoll-betaine and glycerol are present in very substantial amounts and the concentration of myo-inositol is relatively high. A biologically novel, cysteic acid amide (β-sulfoalaninamide) was discovered, but no cysteine and cystine could be found in the free state. This is surprising if one considers the extremely high concentrations of sulfonates; methionine and methionine-sulfoxide are present. Acid-base balance in axoplasm has been established for Loligo and Dosidicus. To obtain more information about the possible significance of these constituents, a comparative analysis of squid blood dialysate has been made by one of us (G.G.J.D.). (Supported by grants B-24 from the N.I.H. and NR 101-100 from the O.N.R.)

3 *Physiology*

NUCLEIC ACID METABOLISM IN TISSUE CULTURE. L. E. Feinendegen,* V. P. Bond, W. W. Shreeve and R. B. Painter. Med. Res. Center, Brookhaven Natl. Lab., Upton, L. I. and Biosciences Div., Battelle Mem. Inst., Columbus, Ohio.

Previous results with HeLa S3 tissue culture cells, using tritiated cytidine with autoradiographic and chemical techniques agreed with the concept that RNA is first synthesized in the nucleus (mainly the chromatin portion) from whence it moves to the cytoplasm. There was also indication that macromolecular RNA in HeLa cells is stable, and that RNA contributes directly to DNA. For the present experiment HeLa S3 cells were labeled with H^3 cytidine for 90 to 120 minutes, and then allowed to grow in regular medium supplemented with carrier. 6 to 72 hours later the nucleic acids were extracted and their total tritium and tritium distribution to uracil and cytosin was determined. The DNA tritium was also evaluated autoradiographically after RNA removal. The results indicate relative stability of macromolecular RNA while the acid soluble RNA renews rapidly. The pattern of DNA labeling indicates in addition direct incorporation into DNA of some intracellular RNA components, not competed for by carrier cytidine, and also utilization of small molecular precursors which had exchanged from one cell to another.

4 *Physiology*

ENDOPLASMIC RETICULUM AND GLUCOSE-6-PHOSPHATASE ACTIVITY. Paul R. Gross, William Pearl*, and Gilles Cousineau*. Dep't. of Biology, New York Univ., and Marine Biol. Lab., Woods Hole.

Glucose-6-phosphatase, which is found in the microsome fraction of normal rat liver cells, may be further localized by treatment of the microsomes with deoxycholate. The DOC insoluble material, consisting mainly of ribosomes, has almost no activity, while most of the recoverable enzyme is found in the DOC-soluble material, derived primarily from membranes of the endoplasmic reticulum. Changes in the relative activity of this enzyme in tumour-bearing and in regenerating liver are well correlated with changes in the morphology of the ER membranes, the latter showing maximum proliferation in cells from well-fed normal animals. In sea urchin eggs, where ER of the type seen in mammalian tissue cells is not found, significant glucose-6-phosphatase activity is nonetheless present. The cytochemical localization of the enzyme in this cell thus becomes a matter of interest in connection with attempts to identify structures homologous with ER. Several possibilities exist among new structures seen in the ground cytoplasm, notably certain smooth-surfaced vesicles. The glucose-6-phosphatase activity resides, however, almost entirely in the yolk fraction of the egg, suggesting that the often-proposed homology between the vesicles and ER requires reexamination. (Supported by grant # A-2302 from the National Institutes of Health, U.S.P.H.S.)

XUM

5 **Physiology**

GLUCOSE UTILIZATION BY RESTING SQUID AXON. J.D. Klingman, M.G. Larrabee and F.D. Carlson (intr. by W.E. Love) Biophysics Dept., Johns Hopkins Univ. and Marine Biological Laboratory, Woods Hole.

Squid nerves, after soaking for 4, 8, or 16 hr in sea-water containing glucose-C-14, were extracted successively with cold acetone, water, ethanol-ether and phenol, leaving a residue. The water extract was separated on a Dow-50 column into a fraction eluted by water and another eluted by 2N-NH4OH. The combined acetone and ethanol-ether extracts ("lipid fraction") and the two water fractions were chromatogrammed unidimensionally on paper which was subsequently scanned for radioactivity. Activity in the water extract was found in 5 major peaks, presumably including aspartate and glutamine or glutamate, and several minor peaks. Most peaks increased progressively with soaking time. Activity in lipid chromatograms was limited to the origin and a small peak nearby. Phenol extracts and residues were both active. No significant activity was extracted from the sheath of one axon°from which axoplasm had been extruded after soaking with glucose-C-14 for 8 hr. (Aided by NIH Grant B-702.)

6 **Biochemistry**

THE STATUS OF SOLUBLE RNA IN MAMMALIAN CELLS. E. L. Hess*, A. M. Herranen and S. E. Lagg. Worc. Fdn. Exp. Biol., Shrewsbury, Mass.

RNA in thymus extracts soluble at 23,000 xg amounts to 260-400 mg./100 gm. wet thymus depending upon tissue and extraction techniques. 95% of this RNA precipitated at pH 5.1. The precipitate contained 11% RNA. Electrophoretic behavior of pH 5.1 precipitate and mixtures of 5.1P and RNA have been studied varying RNA content, pH and ionic strength. Sampling from boundary regions indicated that RNA-protein interactions occurred, with re-equilibration in the boundary layers and bimodal spreading of the boundaries in accord with Gilbert theory. With an excess of protein at pH 7 and u 0.025, RNA existed almost completely in an interacted form whereas at pH 10 and u 0.50 a major portion of the RNA appeared to be unbound. The isoelectric region of the protein was between 4-5 in which region RNA and protein precipitate, whereas below isoelectric conditions a soluble interacted complex existed. Solubility and composition of the complex at pH 3 was a function of ionic strength relationships obtaining while passing through the isoelectric region. Results suggest that soluble RNA in the mammalian cell occurs with proteins as an equilibrium complex in which the forward and reverse velocity constants are functions of pH and ionic strength which obtains within local regions of the cell.

7 **Biochemistry**

SELENIUM-75 IN THE INTRACELLULAR PARTICULATE MATTER OF RAT LIVER. D.M. Roth* and Kenneth P. McConnell. VA Hospital and University of Louisville School of Medicine, Louisville, Kentucky.

Preliminary studies by McConnell et. al. (Nature, 183, 183, 1959) on rat liver cell fractionation at 24 hours showed about one-half and one-third of the total Se-75 activity, expressed as a percentage of the homogenate, in the soluble and mitochondrial fractions respectively, with smaller amounts in the remaining fractions. These studies were extended to include time intervals from 15 minutes to 3 weeks. The total Se-75 activity in the particulate matter at each time interval was greatest in the soluble fraction, with decreasing amounts respectively in the mitochondrial, microsomal and nuclear fractions. The activity in the homogenized liver sample, and in each of the intracellular fractions increased to a peak at about 6 hours, then leveled off to a plateau, followed by a second peak in about a week. Thereafter, activity in all the fractions decreased. The protein-bound Se-75 in each of the fractions was determined by the standard trichloroacetic acid method. Possible theoretical interpretations of these results will be made in relation to Se-75 turnover and protein synthesis. The significance of the incorporation of Se-75 in the ribonucleoprotein of the microsomes will be discussed; and a new technique for counting mitochondria will be described.

8 **Biochemistry**

PROTEIN COMPOSITION OF ZYMOGEN GRANULES. L.J. Greene (Intr. by C.H.W. Hirs)Rockefeller Institute, New York

Zymogen granules were isolated from bovine pancreas and their protein composition determined. The zymogen fraction was obtained by differential centrifugation of the homogenate of fresh pancreas and when examined in the electron microscope was found to consist almost exclusively of zymogen granules. 95% of the protein nitrogen of this fraction was solubilized by dialysis in phosphate buffer. The insoluble material, which was separated by centrifugation, consisted of membranes. The soluble proteins were separated by column chromatography by the procedure of Keller et al. (J. Biol. Chem., 233, 344, (1958)).The identity of the proteolytic enzymes after activation by trypsin was confirmed by assay with synthetic substrates. The chromatograms of the soluble proteins were almost identical to those obtained by Keller et al. for bovine pancreatic juice. This similarity indicates that, as postulated by Heidenhain in 1883, the proteins of the pancreatic juice derive from the zymogen granules.This hypothesis has been confirmed for several enzymes by previous workers; the present study shows that it applies to the total protein content of the zymogen granules.

9 **Biochemistry**

QUANTITATIVE HISTOCHEMISTRY OF THE DEVELOPING HAMSTER TOOTH. ALKALINE PHOSPHATASE AND LACTIC DEHYDROGENASE DISTRIBUTION. Sjoerd L. Bonting and Klaus Nuki * Univ. of Illinois Coll. of Med. and Dent., and Presbyterian-St. Luke's Hosp.,Chicago(Ill.)

The quantitative distribution of alkaline phosphatase (APP) and lactic dehydrogenase (LDH) was determined in ten morphologically distinct zones of the unerupted hamster molar at 0,1,2,3 and 4 days after birth. Fragments, weighing from 10-150 mμg., were obtained by microdissection from frozen-dried 16 μ. serial sections. The fragments were weighed on a quartz fiber "fishpole" balance, and then incubated in suitable substrate media. Substrate turnover was determined microcolorimetrically or fluorimetrically. Results were expressed in moles of substrate split per kg. dry weight per hr. at 37°C (MKH). LDH levels were of the same order in all the cellular zones. Zones furthest from the sites of calcification occupied the lower part of the range. APP activity ranged from 398 MKH in stratum intermedium to 0.44 MKH in the oral epithelium. APP activity increased with age in most zones, exc. in ameloblasts (from 60 to 1.3 MKH) and enamel-dentin (from 16 to 0.59 MKH). Highest APP activity was found in stratum intermedium and subodontoblastic papilla, which are not adjacent to the calcifying enamel and dentin. The high APP levels in stratum intermedium and subodontoblastic papilla form a"barrier" surrounding the actual sites of calcification, thus supporting Neuman and Neuman's theory of calcification. (Supported by US-PHS Grant H-3912).

10 **Biochemistry**

THE LIPIDS OF BOVINE SPERMATOZOA. Lowell D. Miller* Methodius Bartek* and Dennis T. Mayer. Dept. of Agricultural Chemistry. Univ. of Missouri, Columbia.

The lipids of spermatozoa have been implicated in metabolic activities, in fertilization and other reproductive processes. In this study, complex lipid mixtures, extracted with ethanol, ethanol-ether and petroleum ether from washed, freeze-dried bovine spermatozoa, were separated on silicic acid columns. The total lipids comprised 10-14% of the dried cells. The lipid mixture, 300 mgm., was applied to the column and eluted with petroleum ether and increasing amounts of ethyl ether. Phospholipids were eluted with absolute methanol. After chromatography seven distinct fractions, constituting an average of 99.8% of the original lipid mixture, were recovered and characterized. The phospholipid fraction was the largest, about 60% of the total lipids. The combined mono-, di- and triglyceride fractions comprised about 24%, the cholesterol and cholesterol ester fractions totaled some 15%, and saturated hydrocarbons less than 2% of the total lipids.

11 *Biochemistry*

THE DESTRUCTION OF EHRLICH ASCITES TUMOR CELLS BY HEPARIN IN VITRO. Nome Baker*and Max Fields. Radioisotope Service, Veterans Administration Center, and Dept. of Physiological Chem., UCLA Med. Ctr., Los Angeles, California.

Studies have been carried out to determine whether the growth-inhibiting property of heparin on the Ehrlich ascites tumor cell (Lippman, Canc. Res. 17:11, 1957) was due to its action on the nucleus of the cell (Baker, et al., Fed. Proc. 16:149, 1957). Heparin (53 μg/ml) destroyed most of the nuclei of intact cancer cells if cells were first washed with isotonic saline and resuspended in 0.25M sucrose. However, cells of untreated ascites tumor fluid, when diluted ten times in 0.25M sucrose were not destroyed by heparin (530 μg/ml). Extracellular fluid of the tumor contained one or more heat labile, non-dialyzable factor(s), which could prevent the nucleolytic action of heparin. Several materials (other than the factor(s) present in the extracellular fluid) were found which could inhibit significantly the action of heparin on the washed, Ehrlich ascites tumor cell: (a) Ca^{++} in concentrations as low as 3×10^{-6}M; (b) Versene (EDTA) in concentrations as low as 2×10^{-3}M; (c) 0.01M ADP or ATP and (d) 0.15M NaCl or KCl. 1×10^{-2}M Mg^{++} did not protect against heparin. Protective substances also produced drastic morphologic changes in the appearance of the cells and of the nuclei.

12 *Pharmacology*

INFLUENCE OF AGING ON INTRACELLULAR DISTRIBUTION OF CARBONIC ANHYDRASE. Ralph Karler* and Dixon M. Woodbury. Dept. of Pharmacology, Univ. of Utah, Salt Lake City, Utah.

Karler and Woodbury (Fed. Proc. 18: 409, 1959) determined that carbonic anhydrase activity of rat cerebral cortex is divided between two subcellular fractions, mitochondrial and soluble. The study has now been extended to rats of various ages. Two methods were used to fractionate tissue homogenate activity: 1) standard differential centrifugation procedures and 2) the use of a carbonic anhydrase inhibitor in serum. The serum inhibitor identified by Booth (J. Physiol. 91: 474, 1938) was found to inhibit selectively the enzymatic activity of the soluble fraction without affecting the activity of the particulate fraction. Carbonic anhydrase activity was determined by a micro modification of the manometric method of Meldrum and Roughton. The total enzymatic activity of tissue homogenates was found to increase with increasing age. The activity of both particulate and soluble fractions increased with age; however, the activity of the soluble fraction appeared to increase more with age than did that of the particulate fraction. The intracellular distribution results obtained by differential centrifugation and by the serum inhibitor technics were essentially identical. (Supported by Grant B-381, N.I.H. and Nat'l. Neurological Research Found.)

13 *Pharmacology*

FRACTIONATION OF HEPARIN FROM A MAST-CELL TUMOR OF THE MOUSE. J. P. Green, M. Roberts,* and S. M. Day,* Department of Pharmacology, Yale Univ. School of Med., New Haven, Conn.

Heparin from a strain of a murine mast-cell tumor (P-815) has been compared with bovine heparin prepared in the same manner and with heparin obtained from commercial sources. By paper chromatography the murine heparin was resolved into three fractions; the other heparin extracts showed only two components. The unusual fraction was immobile in the solvent system employed. Its concentration was correlated with the anticoagulant activity of the heparin extract and with the levels of 5-hydroxytryptamine (5-HT) and histamine in the mast cell. This fraction could not be eluted from the paper nor could it be eluted from Ecteola. However, it was readily obtained by fractionating heparin preparations with sodium chloride and cetavlon: the unusual heparin precipitated under conditions in which heparins from other sources remained in solution. A heparin with similar chromatographic properties has been tentatively demonstrated in the rat. It is possible that the presence of a unique heparin in the mast cells of the mouse and rat explains the anomalous presence of 5-HT in the mast cells of these species.

14 *Pharmacology*

HEPARIN, HISTAMINE AND 5-HYDROXYTRYPTAMINE IN NEOPLASTIC MAST CELLS IN CULTURE. S. M. Day* and J. P. Green, Dept. of Pharmacology, Yale Univ. School of Med., New Haven, Conn.

The rates of incorporation of glucose-U-C^{14} and glucosamine-1-C^{14} into heparin by two strains of a murine mast cell tumor (P-815) growing in culture have been compared. The larger tetraploid strain of cells showed a greater uptake of glucosamine-1-C^{14} than the near-diploid cells. Neither strain took up glucuronate-6-C^{14}. Glucosamine-1-C^{14} and S^{35}-sulfate in heparin turned over at the same rate. The half-life of heparin was two and a half days. A gradual decrease in the levels of 5-hydroxytryptamine and histamine has been observed in both strains in culture as well as in the mouse. Attempts have been made to increase the levels of these amines by fortifying the culture medium with their precursors as well as the known precursors of heparin. For the same purpose monamine oxidase inhibitors have been added to the culture medium. Parallel experiments have been carried out in vivo.

15 *Pharmacology*

THE EFFECTS OF HYPERTHYROIDISM AND HYPOTHYROIDISM ON THE HEPATOTOXICITY PRODUCED BY CCl_4. Deane N. Calvert* and Theodore M. Brody. Dept. of Pharmacology, University of Michigan Medical School, Ann Arbor.

The authors have proposed that CCl_4 produces its hepatotoxic effects through stimulation of the central sympathetic areas producing constriction of the intrahepatic blood vessels and consequent anoxia of the hepatic parenchymal cells. (Fed. Proc. 18, 375, 1959.) To increase or decrease the tissue oxygen needs thyroxine was administered or thyroidectomy performed upon rats. At 8-10 hours after administration of CCl_4 to animals previously receiving thyroxine the characteristic metabolic changes (disruption of oxidative phosphorylation, Mg ATPase activation) were present to a marked degree. At this time CCl_4 alone produced no such metabolic changes. Conversely, in animals which had been previously thyroidectomized, CCl_4 produced much less marked metabolic changes at 20 hours post-treatment than those animals receiving CCl_4 alone. Catechol amines in the adrenal gland were also measured and were seen to be strongly reduced in the hyperthyroid animals at 10 hours and only slightly reduced at 20 hours in hypothyroid animals. The changes are consistant with a concept of anoxia being the primary event in CCl_4 hepatotoxicity. Supported by USPHS RG 4759-C3.

16 *Pathology*

ELECTRON MICROSCOPIC IDENTIFICATION OF THE FAT AND PROTEIN PARTICLES IN FROZEN-DRIED MOUSE MILK AND LACTATING MAMMARY GLAND. S.R.Wellings,B.W.Grunbaum and K.B.deOme (intr.by H.D. Moon).Dept. of Pathology & Cancer Res.Genetics Lab., Univ. of California, San Francisco & Berkeley, California.

Lactating mouse mammary tissue fixed in osmium tetroxide was prepared for electron microscopic study by standard techniques. There were two sizes of particles in the cells and in the milk within the alveoli: round,dense,homogeneous particles measuring 500-8000mμ.,and smaller particles,also round,but less dense measuring 40-300mμ. Lactating mammary tissue of mice and milk droplets were frozen-dried,embedded in methacrylate under vacuum,thin-sectioned and studied by electron microscopy. These also contained two classes of particles similar to those in the osmium tetroxide-fixed tissue. Frozen-dried thin sections treated with a solution of methanol and ether (1:1) contained only the small particles when examined by electron microscopy. Additional sections of mammary tissue and milk were treated with methanol-ether,fixed in 10% formalin for 10 min.and incubated for 2-12 hrs.in 10% trypsin at pH 7.4. Electron microscopic study of this material revealed that all particles were absent. These observations indicate that the larger particles in osmium tetroxide fixed preparations are fat and the smaller protein.(Supported in part by USPHS CRTY 5029 and ACS grant #P-113).

XUM

17 *Pathology*

CHANGES IN THE NUCLEOPROTEINS OF THE CELL IN NORMAL AND ABNORMAL MITOSIS. Robert Love, Hiroshi Kobayashi* and T. P. Bharadwaj*. Natl. Cancer Institute, Bethesda, Md.

Nucleo-cytoplasmic interchange of several forms of ribonucleoprotein (RNP) has been demonstrated by the toluidine blue-molybdate (TBM) staining procedure (Love and Liles, J. Histochem., 7: 164, 1959). In the Ehrlich and Krebs 2 ascites tumor cells two additional forms of RNP, a diffuse cytoplasmic and a chromosomal form, which can be demonstrated by TBM after very brief fixation, undergo cyclical changes during mitosis. In normal mitosis, granular and diffuse parachromatin accumulates in the nucleoplasm during prophase, at the end of which the granular form is released into the entire cytoplasmic area, while the amorphous form diffuses into the spindle zone. During anaphase amorphous parachromatin diffuses from the chromosomes into the interzonal region. It is suggested that RNA which diffuses into the spindle area constitutes a template for the formation of the spindle proteins. Colchicine produces either metaphase block or prevents the onset of prophase. In the former, the amorphous parachromatin diffuses throughout the entire cytoplasm at the end of prophase and spindle fibers are not formed. When the cell is prevented from entering prophase, parachromatin accumulates in the interphase nucleus and the pars amorpha and the parachromatin (nucleolinus) of the nucleolus increase in size.

18 *Pathology*

CYTOLOGIC RESPONSE OF RAT LIVER TO N-HYDROXY-2-ACETYLAMINOFLUORENE. Henrik A. Hartmann, Elizabeth C. Miller* and James A. Miller*. Univ. of Wisconsin Med. School, Madison, Wis.

Alterations of cytological components were compared in livers from male rats receiving 2-acetylaminofluorene (AAF) or a recently discovered major metabolite of this carcinogen, N-hydroxy-2-acetylaminofluorene (N-HO-AAF) (Cramer, Miller and Miller, J. Biol. Chem., March 1960). These components (45 μ-moles per dose) were injected i.p. 3 times per week. After 7 doses of N-HO-AAF some rats had to be sacrificed whereas the rats receiving AAF were in good condition. N-HO-AAF produced a striking severe hydropic degeneration which involved all zones of the liver lobules. The hydropic vacuoles did not stain with Sudan III. Because of their uniformity in size and distribution they may represent swollen mitochondria. The cytoplasm showed a loss of basophilic staining material which may be due to reduction or alteration in dispersion of RNA. Proliferation of bile ducts was prominent at this time with invasion of the periphery of the lobule. After 3 to 4 weeks on N-HO-AAF bile duct proliferation was much more extensive and often reached the central zones of the lobules. The lack of orderly arrangement of the liver trabeculi suggested regenerating liver nodules. The hydropic swelling at this time was still evident in the peripheral zones. The animals receiving AAF showed only scattered loss of basophilic material.

19 *Pathology*

EFFECTS OF CYTOXAN ON DNA SYNTHESIS AND THE MITOTIC CYCLE IN L4946 MOUSE LEUKEMIA. Jesse E. Sisken,* Ervin Kovacs* and Riojun Kinosita. City of Hope Med. Ctr., Duarte, Calif.

Cytoxan (3.2 mg/20 gm mouse) was injected intramuscularly into AKR mice approximately 72 hours after tumor implantation. Before and after drug administration, tritiated thymidine ($T\text{-}H^3$), a specific label for DNA, was injected intraperitoneally, 10-20 μc/mouse. Tumor samples were removed periodically, smeared, stained with Feulgen, and autoradiographs prepared. Data indicate that Cytoxan administered 30 minutes before or after $T\text{-}H^3$ did not affect the percentage of cells synthesizing DNA, the amount of label per cell or the progression of labeled cells into mitosis. When $T\text{-}H^3$ was injected 6 hours after Cytoxan, the percentage of interphase cells becoming labeled was not decreased but the amount of label per cell was much lower and progression of labeled cells into mitosis was halted. Although the drug is radiomimetic cytologically, the labeling characteristics of interphase cells after the 6-hour delay seems at variance with previous studies on X-ray effects, which showed that even low doses will decrease the number of cells entering DNA synthesis (Howard and Pelc, Heredity, suppl. 6:261, 1953; Lajtha et al, Radiation Research, 8:1, 1958). (Supported by PHS grant 4159.)

20 *Immunology*

RE FUNCTION AND PHOSPHATASE ACTIVITY IN MOUSE LIVER. G.J. Thorbecke, L.J. Old, B. Benacerraf, and D.A. Clarke. N.Y.U. College of Med., New York and Sloan Kettering Institute, Walker Lab., Rye, N.Y.

A histochemical study of acid and alkaline phosphatase activity by the Gomori technique has been made in mouse liver following procedures known to influence RES function. In normal liver, acid phosphatase activity is found almost exclusively in Kupffer cells. Alkaline phosphatase activity is very low and is confined to the walls of large vessels. Characteristic alterations occur under conditions where phagocytic function is increased and proliferation of RE elements is observed. Multiple endotoxin or zymosan injections, BCG infection, growth of transplanted sarcoma 180 or Friend virus leukemia, all cause similar changes which consist of an increase in acid phosphatase activity due to the presence of increased numbers of phagocytic elements and a striking appearance of alkaline phosphatase activity in the lining of sinusoids throughout the liver. Liver homogenates were analysed for enzyme activity following zymosan or BCG. Acid phosphatase activity was increased up to 2 fold and alkaline phosphatase activity 3-5 fold. The first change seen appearing as early as 2-5 hours following a single injection of endotoxin, is a patchy increase in alkaline phosphatase activity in sinusoidal linings.

079. CELLS–Membranes

Physiology

TRANSPORT PHENOMENA IN MUSCLE STUDIED BY THE USE OF RAT DIAPHRAGM AS A "MEMBRANE". G. Ungar and D. V. Romano*, Dept. of Pharmacology, U. S. Vitamin Corp., New York.

Fragments of diaphragm muscle with intact nerve supply were interposed as a barrier between oxygenated Ringer's solution on one side and isotonic sucrose or choline Cl on the other. Rates of transport of Na^+ and K^+ were measured across resting and stimulated muscle. During excitation, rate of passage of Na^+ decreased while that of K^+ increased. This parallels previous results indicating fall of Na^+ activity and rise of K^+ activity in stimulated structures (Cytologia, in press). Insulin (which lowers excitability) causes considerable increase in the transport of Na^+ across the muscle while K^+ transport shows little change. Transport of other cations, anions and sugars is being investigated. The results, on the whole, are consistent with the hypothesis of adsorption, probably dependent on the configuration of cell proteins (J. Gen. Physiol., 40:635, 1957; Proc. Soc. Exp. Biol. & Med., 97:324, 1958). Stimulation may act primarily by rearranging certain protein molecules and modifying their adsorption properties. These, in turn, control ionic activities and perhaps also the binding of water. Insulin may produce similar but opposite changes (Nature, 183:49, 1959).

2 *Physiology*

PENETRATION OF SURVIVING EXCITABLE AXONS BY PROTEASES AND EFFECTS OF PROTEASES AND PHOSPHOLIPASES ON SO-CALLED MEMBRANE RESISTANCE AND CAPACITY, Julian Tobias, Dep't. of Physiology, University of Chicago, Chicago, Ill.

Squid axons were suspended in catenary form with dependent centers submerged in sea water with or without trypsin and/or chymotrypsin. After several hours the axons were removed, washed and shown excitable. Axoplasm was extruded and assayed for protease activity on gelatin films. Axoplasm from experimental axons but not from controls was proteolytic. Apparently large protease molecules can penetrate the surface including the hypothetical membrane without producing inexcitability. Controls make it unlikely that entrance was through cut branches.

Length constant, membrane resistance an capacity, and axoplasmic resistance were measured on giant axons from the lobster nerve cord by the method of Hodgkin and Rushton (Proc.Roy. Soc.Lon. B,1946,133,p.444) as a function of time, in controls and in axons exposed to proteases or phospholipases. Proteases have small effects, if any, on resistance but may lower it slightly. No effect was d tected on capacity or on axoplasmic resistance. Membrane resistance can fall markedly simply as a function of time though the axons remain functional. Phospholipases produce a precipitous fall in resistance and capacity and render the axon inexcitable. The data indicate that the high impedance surface element is lipid.

3 **Physiology**

The Absence of a Sodium Equilibrium Potential in Excitation Currents of a Small Isolated Region of a Frog Muscle Membrane A1 Strickholm (intr. by N.Brewer) Dept. of Physiology, Univ. of Chicago, Chicago, Illinois

A small surface region (5 to 40 microns dia.) of a frog muscle fiber is isolated electrically by placing a smooth tipped micro electrode against the cell surface. Depolarizing the region beyond 30 mV. initiates transient excitation currents with peak obtainable currents up to 5×10^{-3} a/cm^2 and maximum integrated current flow equivalent to one unit charge per 100-200 Å^2 of surface area. The excitation currents are always positive inward at membrane depolarizations measured up to 1/2 volt. No excitation current reversals are seen at surface depolarizations producing transmembrane potentials equal to and beyond the sodium equilibrium potential. The excitation currents are dependent upon external sodium and show no detectable outward potassium current phase as in the squid axon. However, voltage stressing a region for a few msec. at ± 1/2 volt or less produces inexcitability which can be reversed by applying a constant hyperpolarizing voltage bias of a few to over 100 mV. This restored region now exhibits behavior analogous to the voltage clamped squid axon and excitation currents become zero at transmembrane potentials near the sodium equilibrium potential and reverse direction with further depolarization. Also appearing now is a delayed outward current component.

4 **Physiology**

RECTIFICATION IN MUSCLE MEMBRANE. W.H. Freygang Jr. and R.H. Adrian*. National Institutes of Health, Bethesda, Md. and The Physiological Laboratory, Cambridge, England.

Three micropipettes were inserted into a fiber of the frog sartorius muscle. The pipettes were 0.44 mm. apart and one was 0.44 mm. from the end of the fiber. Constant current pulses were injected through the pipette farthest from the end; the other two pipettes measured the membrane potential. With this arrangement the electrotonic potential is nearly constant along the end part of the fiber. The membrane conductance was calculated from the difference between the potentials at the two voltage-recording pipettes together with the potential at one of them. Substitution of choline for Na and SO_4 for Cl made it possible to estimate individual ionic conductances in solutions with normal and high K concentrations. The results show that K-conductance is reduced with an outward flow of K ions and is increased with an inward flow. The presence of Cl raises the total conductance and the Cl-conductance is less sensitive to the direction of current flow. When this conductance data is converted into constant-field permeability coefficients, the results are consistent with those obtained by other methods (Hodgkin and Horowicz, J. Physiol. 148: 127, 1959).

5 **Physiology**

CILIARY REVERSAL AND ACTIVATION BY ELECTRIC CURRENT; THE LUDLOFF PHENOMENON IN TERMS OF CORE AND VOLUME CONDUCTORS. Theodore L. Jahn, University of California, Los Angeles.

In Paramecium there is no known correlation between the direction of electric current through the membrane and of ciliary beat. The Ludloff (1895) phenomenon, an anodal shift in the limit of the area of reversal with increased current strength, has seemed contradictory to most other data. However, by assuming Paramecium to be a core conductor immersed in a volume conductor and by applying the laws of polarizing currents it is possible to explain all data on reversal of normal ciliary action, and also on activation of cilia in immobilized specimens. It is assumed that a threshold degree of depolarization of the normal membrane potential causes reversal. The Ludloff phenomenon is caused by anodal progression of this degree of depolarization with increasing membrane current. If it is also assumed that an increase in the membrane potential of immobilized specimens causes activation in the normal direction, one can predict anodal activation, progression of reversal with decrement in velocity, time course of development of excitation, anodal stimulation upon "break", stimulation by linearly rising currents, relative refractory and supernormal periods, effect of angle of orientation, and effect of ACh and anti-AChE. Assumption of a neuromotor system is not needed.

6 **Physiology**

CHANGES IN THE COMPOSITION OF SALIVA PRODUCED BY ADRENALIN AND INSULIN. K. Martin (intr. by A.S.V. Burgen.) Dept. of Physiology, McGill Univ., Montreal, Canada.

It was shown by Stavraky that administration of large doses of adrenalin to the cat led to changes in permeability of the submaxillary gland so that glucose penetrated freely into the saliva and the concentration of sodium and chloride increased considerably. It has been found that this effect also occurs in the dog submaxillary and parotid glands and can be produced by local injection of adrenalin into the artery to the gland. Noradrenalin in comparable doses is ineffective. The effect of adrenalin can be prevented by previous treatment by dibenzyline but is not reversed if already present. Insulin in large doses (administered with glucose to prevent hypoglycaemia) abolished the effects of adrenalin. Adrenalin increases the concentration of amino acids and non-electrolytes in saliva and enables sucrose to penetrate into saliva which it cannot normally do. Studies with radioactive tracers have shown that changes in function of the salivary tubules are produced by adrenalin. It is concluded that adrenalin causes an increase in the permeability of salivary glands. Studies of the influence of adrenalin on the salivary concentration of electrolytes show that these are effected selectively. (Supported by a grant from the National Research Council of Canada.)

7 **Physiology**

EFFECT OF CO_2 AT BENZENE-WATER INTERFACES. Sears, D. F. and R. M. Eisenberg. (intr. by L. M. N. Bach). Department of Physiology, Tulane School of Medicine, Tulane University, New Orleans, La.

Interfacial tensions between benzene and water have been determined for 2.5 to 40 mEq per liter solutions of NaCl, KCl, $NaHCO_3$, and $KHCO_3$. The benzene phase contained 5 volumes percent oleic acid with which these salts could react or form oleate soaps. Chloride salts produced little change in interfacial tensions (-2 dynes per cm.) from that corresponding to the tension measured between pure water and benzene with 5% oleic acid. Bicarbonates produced progressive decreases in interfacial tension as their concentration increased in the aqueous phase. Equilibration of the benzene phase with 100% CO_2 caused no change in interfacial tensions with chloride salts from that determined in the absence of CO_2. However, addition of CO_2 caused marked increases in interfacial tensions with bicarbonates. Values approached those obtained for the chlorides. The action of CO_2 was discussed in relation to its ability to reverse oil-in-water emulsions to water-in-oil.

8 **Biochemistry**

COMPLEX LIPIDS OF CELL MEMBRANES. Peter S. Olmsted (intr. by H. W. Robinson), Temple Univ. School of Med., Phila., Pa.

Cell membranes are rich in lipids and proteins, but may also contain carbohydrate or other moities as well, all of them linked together to form various glycoproteolipids. To obtain these complex lipid-soluble substances in sufficient quantity from membranes human red blood cell ghosts were utilized as starting material. They were extracted with $CHCl_3$:CH_3OH (2:1) and the extract dialyzed twice against dil. buffered salt solns. to remove water sol. compounds. The sac contents were then centrifuged and the organic phase dialyzed repeatedly against $CHCl_3$:CH_3OH (2:1) to remove small lipid soluble compounds. Control experiments indicated the complete dialyzability of triglycerides, lecithin, cholesterol and cholesterol esters. The extract was dried, taken up in Skelly B and chromatographed on aluminum silicate columns with a gradient elution procedure using increasingly polar solvents (Skelly B, $CHCl_3$, CH_3OH and H_2O). Twenty-five peaks were obtained and analyzed. The peaks differed widely in their content of P, carbohydrate, ester, sialic acid, and protein. The homogeneity of these chromatographic peaks is being investigated, but it appears probable that a variety of substances exhibiting lipid solubilities and containing covalently linked non-lipid moities are present in human erythrocyte membranes.

XUM

9 *Pharmacology*

A MECHANISM OF PERMEABILITY CHANGE IN LIVING MEMBRANES. A.M. Shanes, National Institutes of Health, Bethesda, Md.

It was noted recently (Shanes, Pharmacol. Rev. 10:140, 1958) that the exact parallelism between the blocking potency of stabilizers (e.g., local anesthetics, alcohols) and their ability to increase "spreading pressure" in monomolecular lipid films may be due to entry of such compounds into the region of the membrane surrounding sites of ion passage during excitation; this would interfere with displacement of membrane molecules necessary for increase in ion permeability during excitation. Voltage-clamp experiments are consistent with this view (Shanes et al., J. Gen. Physiol. 42:793, 1959). The proposed role of spreading pressure in permeability is supported by (1) the increase in ion permeability caused by "unstabilizers" or "labilizers" (e.g. veratrum alkaloids in veratrine), which are found to remove film (and membrane?) molecules and (2) the antagonism to the alkaloids exerted by calcium, low pH, and stabilizers in both films and living membranes (Gershfeld and Shanes, Science 129:1427 and J. Physiol. 148:57P, 1959). It will now be shown that the same concepts apply to drug-induced negative afterpotentials; moreover, "electroadsorption" provides a similar basis for the permeability changes accompanying alterations in membrane potential.

10 *Pharmacology*

EFFECTS OF CONCENTRATION GRADIENTS ON THE POTENTIAL DROP ACROSS THE SMALL INTESTINE OF THE DOG. A. C. Brown,* E. D. Jacobson,* K. Hubel* and R. L. Vick. Dept. of Physiology, State Univ. of New York, Syracuse, New York.

An 8 cm. segment of proximal jejunum with intact blood supply was perfused with isosmotic solutions containing varying concentrations of sodium and chloride. The potential drop between the intestinal lumen and the venous blood was measured. For a given composition of perfusing solution the p.d. remained at a constant level for up to 5 hours. When the segment was perfused with isosmotic NaCl solution the p.d. across the intestine was ca. 5 mv. (lumen negative). When the Na of the perfusion fluid was replaced by choline, the lumen became less negative; when no Na was in the inflowing solution, the p.d. was near zero. Substitution of sulfate for chloride caused the lumen to become increasingly negative. Solutions in which all of the Cl had been replaced by SO_4 produced a three-fold increase in lumen negativity. Simultaneous reduction of both Na and Cl (replacement of sucrose) gave potentials similar to those observed when only Cl was decreased. It appears that the permeability of the dog jejunal mucosa to chloride is greater than its permeability to sodium. The presence of a voltage gradient in the absence of concentration gradients implies active transport of sodium out of the lumen or of chloride into the lumen.

11 *Pharmacology*

DISSOCIATION OF CONTRACTION AND POTASSIUM EFFLUX IN SMOOTH MUSCLE. Leon Hurwitz, Betty Tinsley* and Frank Battle*. Dept. of Pharmacology, Vanderbilt Univ. School of Med., Nashville, Tenn.

Normally, a pilocarpine induced contraction of the isolated guinea pig ileum is associated with a change in potassium transport across the cell membranes. Bathing the tissue in a Ca-free medium will effect a dissociation between contractile response and potassium efflux. Initially, the changeover from normal Tyrode's solution to a Ca-free medium will elicit a slight transient increase in smooth muscle tone followed by a return to complete relaxation. Within an hour the contractile response to pilocarpine becomes negligible. Conversely, the efflux of potassium (measured by means of the radioactive tracer K^{42}) will undergo a sudden rise in magnitude and continue to stay above normal. The administration of pilocarpine, after the tissue has been bathed in the Ca-free solution for one or two hours, will evoke a moderate additional increase in potassium efflux. Replacement of Ca ion for an equimolar quantity of Mg ion accentuates the elimination of the contractile response to pilocarpine. Here again, the potassium efflux of the relaxed tissue rises above normal and responds to pilocarpine with a further increase in magnitude. Thus pilocarpine can modify potassium transport in the isolated ileum irrespective of its effect on smooth muscle tension.

12 *Pharmacology*

PENETRATION OF DRUGS INTO ERYTHROCYTES. Lewis S. Schanker and Panayotis A. Nafpliotis*. Natl. Insts. of Health, Bethesda, Md.

An investigation of the mechanisms by which drugs enter various tissue cells has been initiated by a study of the permeability of erythrocytes to organic compounds. Human erythrocytes were suspended in Tyrode's solution (pH 7.4) containing 1-4 mmol/l of a drug, and the suspensions shaken in an incubator at 37°C. After various intervals, the cells were isolated by centrifugation, and analyzed for the drug. A number of organic bases entered the cells at rates roughly related to their lipid/water partition ratios at pH 7.4. For example, weak organic bases with high lipid-solubilities (e.g. aniline) penetrated very rapidly; those with relatively low lipid-solubilities, like serotonin, epinephrine and norepinephrine, penetrated at slower rates; and quaternary ammonium compounds, which have very low lipid-solubilities, entered at very slow rates. These observations are consistent with the hypothesis that the cell membrane is lipoid in character and is passively penetrated by lipid-soluble substances. Preliminary results with several sulfonic acids suggest that the penetration of these anions resembles that of the inorganic anions in that they enter the cell much more rapidly than do cations.

13 *Pharmacology*

DRUG EFFECTS ON THE RADIORUBIDIUM FLUX THROUGH ISOLATED RABBIT MESENTERY. W. O. Berndt* and R. E. Gosselin. Department of Pharmacology and Toxicology, Dartmouth Medical School, Hanover, New Hampshire.

The movement of Rb^{86} across the isolated rabbit mesentery exhibits the kinetics of passive diffusion. The effects of drugs on this process can be shown by changes in the first order rate constant k when the source concentration is held constant and the sink concentration is allowed to rise. Control experiments showed that in terms of k the membrane does not deteriorate over a 5-hour period. Temperature studies yielded a Q_{10} value of about 1.6 (28°-38° C.). Each drug was tested at an in vitro concentration of 10 µg/ml (free base). In a modified Krebs-Ringer-phosphate, epinephrine bitartrate and acetylcholine iodide had no effect on the Rb^{86} flux at 38° C. Histamine dihydrochloride caused a 28% increase in the k value and 5-hydroxytryptamine creatinine sulfate (5-HT) produced a 17% rise in k. At 22° C. histamine had no effect, epinephrine a questionable effect and 5-HT a distinct effect, although the results were more variable than at 38° C. In similar trials with Krebs-Ringer-bicarbonate at 38° C., 5-HT increased k by 57%, histamine by 39% and acetylcholine by 51%. (Supported by PHS research grant RG 5145.)

14 *Pharmacology*

CORRELATION OF THE METABOLIC EFFECTS OF BENZALKONIUM CHLORIDE WITH ITS MEMBRANE EFFECTS IN YEAST. (INTR. BY P. K. KNOEFEL.) THOMAS G. SCHARFF, DEPT. OF PHARMACOLOGY, UNIVERSITY OF LOUISVILLE, SCHOOL OF MEDICINE, LOUISVILLE, KY.

AT PH 5.5 VARYING AMOUNTS (0-16 MGM/GM. YEAST) OF THE SURFACTANT, BENZALKONIUM CHLORIDE, WERE ADDED TO NONPROLIFERATING SUSPENSIONS OF BAKER'S YEAST. MEASUREMENTS WERE MADE OF (A) NET POTASSIUM LOSS, (B) PERCENT CELLS STAINED WITH NILE BLUE (IN THE PRESENCE OF CALCIUM CHLORIDE WHICH PREVENTS STAINING BY DYE ALONE), (C) RATE OF ANAEROBIC CO_2 PRODUCTION, (D) RATE OF ANAEROBIC GLUCOSE UTILIZATION, AND (E) DECARBOXYLATION OF PYRUVATE. THE PERCENTAGE OF INHIBITION OF (C) AND (D), THE % OF CELLS STAINED, AND THE % OF K+ LOSS WERE IN APPROXIMATE 1-1 RATIOS. THE NET % OF K+ LOSS AND THE ABILITY TO DECARBOXYLATE PYRUVATE WERE DIRECTLY RELATED. IT IS CONCLUDED THAT THESE METABOLIC AND MEMBRANE RESPONSES TO SURFACTANT, ARE ALL-OR-NONE RESPONSES, I.E., WITH INCREASING SURFACTANT CONCENTRATION GREATER NUMBERS OF CELLS SIMULTANEOUSLY LOSE ALL OR NEARLY ALL OF THEIR K+, CEASE FERMENTING, AND DECARBOXYLATE PYRUVATE. (SUPPORTED BY U.S.P.H.S. GRANT, NO. E-2234)

080. CELLS–Microsomes

Physiology

1

DEPRESSION OF GLUCOSE-6-PHOSPHATASE ACTICITY IN CARBON TETRACHLORIDE FATTY LIVER. R. O.RECKNAGEL* (intr. by F. R. Mautz) Dept. of Physiology, Western Reserve University, Cleveland 6, Ohio.

G-6-Pase activity of isotonic sucrose homogenates of liver was determined in rats fed CCl_4. Activity was at control levels 2 hr post CCl_4 feeding, but was only 59% of control levels at 4 hr. Activities as % of control levels, at later times, were: 6 hr, 50; 10 hr, 34; 16 hr, 41; 20 hr, 28. These data establish the early loss from liver of a microsomal enzyme following CCl_4 poisoning, and suggest that changes in the endoplasmic reticulum are an early feature of the developing pathology. In other experiments, 4 hr post CCl_4 feeding, G-6-Pase was depressed 52%, whereas no activation of the latent acid phosphatase of the lysosomes had occurred. G-6-Pase activity of dilute whole liver homogenates increased 29 to 95% above control levels following incubation in vitro at 0-1° in isotonic sucrose containing CCl_4. At 30°, 81 to 92% of the enzyme was lost in 20 min. At 15 to 50 eq mg of isolated microsomes per ml of isotonic sucrose, all G-6-Pase activity was lost in 20 min at 30°. (Supported by U.S.P.H.S. Grant No. A-1489(C2) and a U.S.P.H.S. Senior Research Fellowship-SF-163).

Biochemistry

2

HYPERFUNCTION IN RAT LIVER MICROSOMES BY CORAMINE. Fred G. Brazda and Ralph W. Baucum*. L. S. U. Sch. of Med., New Orleans, La.

Pretreatment of rats with coramine results in markedly shortened "sleeping time" after injection of pentobarbital. In addition, it tends almost to eliminate the sex difference ordinarily shown by adult animals. Liver microsomes and soluble fraction from treated animals are able to metabolize pentobarbital in the Brodie system several times as rapidly as do control tissues. Use of other diethyl carboxamides (diethyl benzamide, coramine methochloride, diethyl butyramide) in this manner also results in shortened sleeping time in weanling rats following pentobarbital injection. Thus, the induction of enhanced enzymatic activity in the liver microsomes of the rat for the oxidation of pentobarbital may be brought about by the action of a variety of diethyl carboxamides. In the series tested the influence of the group attached to the carboxamide is of varied significance. The fact that this induction may be brought about by molecules structurally quite unlike the substrate is of particular interest. Other, related compounds are being studied to determine the importance of structural modifications. (* Louisiana Division, American Cancer Society Medical Student Summer Research Fellow.)

Biochemistry

3

STUDIES ON DRUG DETOXICATION BY RAT LIVER MICROSOMES. Jay S. Roth and Josef Bukovsky * Hahnemann Med. Coll.,Phila.

Some biochemical aspects of the enzyme system in rat liver capable of N-demethylation of narcotic drugs have been studied. Methods for preparation of the active fractions, combined microsomes and supernatant (PS), and microsomes (P), as well as the conditions of the assay have been investigated. To obtain maximum activity, minimal homogenization with a loose-fitting homogenizer is essential; 0.3 M sodium phosphate buffer, pH 7.3, is most effective and the substrate concentration must be increased to 20 μmoles when PS from 0.5 g. of liver is used. Under optimum conditions, 5-8 times the activity reported by Axelrod (J.Pharm.& Exper. Therap. 117,322, 1956) may be obtained with PS. Starvation of rats for 16-18 hrs. completely abolishes demethylation by PS. This may be due to depletion of glucose-6-phosphate (G6P) since increasing levels of G6P or G1P restore activity to control values. However, P prepared from starved rat liver are strongly inhibitory to demethylation by PS from fed rats, and this inhibition is not reversed by addition of glucose phosphates. Properties of the inhibitor associated with P from starved rats will be presented as well as a discussion of the implications of the findings for drug detoxication in vivo. (Supported by grants from the U.S.A.E.C. and N.I.H.)

Biochemistry

4

PHYSICAL AND CHEMICAL STUDIES ON THE PROTEINS OF YEAST RIBOSOMES. Fay Hoh Yin* and Robert M. Bock, Dept. of Biochemistry, Univ. of Wisconsin, Madison.

The ribosomes of yeast may be obtained as a component which sediments as a single peak in the ultracentrifuge. The sedimentation and diffusion coefficients ($S_{20,w} = 82 \times 10^{-13}$ sec^{-1}, $D_{20,w} = 1.4 \times 10^{-7}$ cm^2 sec^{-1}) gave a molecular weight of 4.6 million ($\bar{V} = 0.67$). The data from sedimentation, diffusion, low angle x-ray scattering and electron microscopy are best fitted by a porous spherical model with 1.33 grams of water of hydration per gram of ribosome. The composition of the ribosome is 43% RNA, and 57% protein. Base, acid, urea, 67% glacial acetic, low and high salts, detergents, thioglycollate and formamide failed to give a stable solution of the ribosomal protein. We found that, after removal of the RNA with ribonuclease, a simultaneous treatment with 8 molar urea and 0.2% Na Dodecyl sulfate gave a solution which remained stable even after removal of the urea and detergent by dialysis. Velocity and equilibrium ultracentrifugation showed that the ribosomal protein is heterogeneous with an average molecular weight of 12,000. Eight different amino acids were found as amino terminal groups. If this protein molecule is a structural unit of the ribosome, then 230 of the 12,000 molecular weight sub-units could occur in each 82 S ribosome.

Pharmacology

5

LIVER MICROSOMAL DRUG-ENZYME ACTIVITY AFTER STARVATION. Robert L. Dixon* and James R. Fouts. Dept. Pharmacology, State Univ. of Iowa, Iowa City.

Electron micrographs detail a number of changes in hepatic microsome structure resulting from starvation. Such changes resemble those we have already shown to be associated with a marked depression of drug metabolism in this subcellular fraction. Male mice were starved for 36 hours and then given 80 mg/kg hexobarbital intraperitoneally. Unstarved mice slept 9.6±2.2 minutes while starved mice slept up to 80 minutes and more. Starved mice were grouped according to their sleeping times and 12 hours later metabolism of a variety of drugs by 9000 x g supernates of pooled liver samples from each group was determined in vitro. Compared to normal livers all oxidative pathways of metabolism were markedly lowered while reductive pathways were unaffected. We have shown also that the administration of hexobarbital 12 hours before the in vitro assays had no effect on the results. Thus, in 12 hours the dose of hexobarbital had not led to measurable induction of enzyme formation in these mice. Twenty-four hours after allowing the starved animals to have access to stock feed, drug enzyme activity was back to normal. Diet therefore becomes another factor which may affect the activity of these microsomal drug-enzymes and should be controlled in experiments where drug effects are quantitated. Supported by USPHS.

Pharmacology

6

PHYSIOLOGICAL DISTRIBUTION AND METABOLISM OF IMIPRAMINE (TOFRANIL). J.R. Gillette, J.V. Dingell* and G.P. Quinn. Natl. Insts. of Health, Bethesda, Md. and Geigy Chem. Corp., Ardsley, N.Y.

Imipramine is a new antidepressant drug for the treatment of mental disorders, but differs from other antidepressant drugs in that it is not a monoamine oxidase inhibitor or a stimulant. Its slow onset of action in man may be due to an irreversible reaction at its receptor site, to the accumulation of the drug in the body, or to the accumulation of an active metabolite. To study this problem a sensitive and specific fluorometric method for its estimation has been developed. Preliminary studies in rabbit show that the drug readily enters brain and is highly localized in various tissues. Despite its localization, the drug almost completely disappears from the body in 6-8 hours. Similar results were independently obtained by Hermann and Pulver, who used a less specific and less sensitive method than the one employed in these studies (see Hafliger, Can. Psych. Assoc. J. 4, S69, 1959). Little drug is excreted unchanged, but metabolic products are present in the urine. Experiments with rabbit liver preparations show that imipramine is metabolized by an enzyme system in microsomes that requires TPNH and oxygen. Studies are underway to determine if man also rapidly metabolizes the drug.

XUM

081. CELLS–Mitochondria

Physiology

MITOCHONDRIAL COUNTS BASED ON OSMOTIC SWELLING. A.T. Miller, Jr. and L.D. Gunn.* Univ. of N.C. Med. School, Chapel Hill.

For purposes of counting, mitochondria are commonly distinguished from other cytoplasmic granules by their property of swelling in hypotonic media. The variability of data in the literature led us to investigate the swelling of mitochondria in media of varying osmotic concentrations (0.125 M sucrose, 0.0625 M sucrose and distilled water). It was found that with increasing hypotonicity there was an increase in the proportion of granules showing swelling as well as in the degree of swelling of individual granules. This could not be explained by a slower rate of swelling in the more concentrated media, and apparently indicates a graded resistance to osmotic swelling in the mitochondrial population. We therefore recommend distilled water as the diluting medium for mitochondrial counts. In both homogenates and washed "mitochondrial" preparations, only 35-40% of the granules swelled in distilled water. Enzyme assays indicated virtually complete removal of microsomes by 3 washings, and the identity of the non-swelling granules has not been established. If they are mitochondria, osmotic swelling is an inadequate criterion for identification; if they are not mitochondria, the conventional washing procedure appears to be inadequate for the preparation of pure mitochondrial fractions. (Supported by N.I.H. Grant C-3996)

2 *Biochemistry*

INFLUENCE OF FATTY ACID IN DIET ON MITOCHONDRIA. M.K. Horwitt, B. Century*, C.C. Harvey* and F. Navazio.* Mendel Res. Lab., Elgin State Hospital, Elgin, Illinois.

That depot fats can take on some of the characteristics of ingested fat has long been recognized but the concept that ingested fatty acids can alter the composition of subcellular particles is a more recent development. Altering the fatty acid content of the diet by feeding coconut oil or corn oil produced mitochondrial lipids in brain and other tissues which are markedly different in fatty acid composition. For example, calculating the amounts of myristic, linoleic, and C_{20}-triene present in chick brain mitochondria as percentages of oleic acid present after feeding coconut oil diets, gave 4, 2, and 28%, respectively, and 2, 8, and 6%, respectively, on corn oil diets. The amounts of tocopherol provided do not seem to affect the levels of fatty acids deposited in the lipoproteins but the amounts of tocopherol required to prevent pathology was related to the level of polyunsaturated fatty acids deposited.

3 *Biochemistry*

OSMOTIC REVERSAL OF MITOCHONDRIAL SWELLING. Henry Tedeschi (intr. by D. L. Harris.) College and Department of Physiology, University of Chicago, Chicago, Ill.

It is known that mitochondria follow osmotic law over a wide range of concentrations of external medium. Accordingly, they can swell reversibly when suspended in hypotonic media or in solutions of a penetrant (Tedeschi and Harris, Arch. Biochem. 58: 52 (1955)). Changes in volume, which are as yet largely undefined, also occur. Swelling of mitochondria induced by the in vitro addition of thyroxine or exposure to solutions of mannitol, sucrose or potassium chloride, can be reversed completely or almost completely by osmotic means. From these results, it would seem most probable that the underlying phenomenon is osmotic in nature. In some of these cases, without the addition of thyroxine, it has been shown that the swelling is paralleled by the penetration of the solute (Jackson and Pace, J. Gen. Physiol. 40: 47 (1956)). Therefore, it is not unlikely that, at least in these cases, we are dealing with a permeability phenomenon and not an independent swelling. (Aided by a grant of the American Cancer Society.)

4 *Biochemistry*

RELATIONSHIP OF SUBSTRATE OXIDATION TO PROMOTION AND PREVENTION OF SWELLING OF ISOLATED RAT LIVER MITOCHONDRIA. F. Edmund Hunter, Jr., H. B. Burch, J. Fink*, and L. Ford*. Pharmacology Department, Washington University, St. Louis.

Most substrates promote swelling, possibly because of changes related to electron transport. However, pyruvate and oxalacetate, which also are substrates, prevent swelling at 2-5 mM. Under conditions used, pyruvate also blocks the swelling-producing effect of phosphate, succinate, and β-hydroxybutyrate. This protective effect probably is direct and not related to function as a substrate. It may result from complex formation with a group in the mitochondrial membrane. Low endogenous substrate may be the primary basis for decreased spontaneous or phosphate-induced swelling of mitochondria from different tissues and from the same tissue in different circumstances. Mitochondria from riboflavin deficient rats, known to be resistant to swelling, behave much like normal mitochondria with added substrate, especially if a low concentration of phosphate is present. Incomplete return to a normal swelling rate may be related to the lower substrate oxidation rate in such mitochondria. The lysis of mitochondria by 0.2 mM ascorbate represents an action different from increased permeability, for typical lysis by ascorbate occurs after phosphate-induced swelling is complete. (Supported by USPHS grants C-2284 and A-1566.)

5 *Biochemistry*

INHIBITION OF SPONTANEOUS AND INDUCED SWELLING OF MITOCHONDRIA BY SPERMINE. Edward J. Herbst and Barbara H. Witherspoon* Univ. of Md. School of Med., Baltimore, Md.

Spontaneous swelling of rat liver mitochondria in 0.25 M sucrose-0.02 M Tris, pH 7.4 is prevented by 0.05 mM spermine and significant protection against swelling is afforded by 0.01 mM spermine. Spermidine and other polyamines also inhibited swelling but diamines were either ineffective or only slightly effective at much higher concentrations. The most active compounds were spermine and spermidine which occur in liver at concentrations equivalent to the effective levels in these experiments with isolated mitochondria. Amines were also tested as antagonists of mitochondrial swelling in the presence of the inducers β-hydroxybutyrate, succinate, glutamate, thyroxine, Ca^{++}, phosphate, glutathione, ascorbic acid, arsenite, and oleate. 0.1 mM spermine inhibited swelling in all systems while other polyamines were less effective inhibitors of induced swelling. 0.5 to 1 mM spermine produces a visible aggregation of mitochondria in sucrose-Tris and the aggregates sediment at low centrifugal forces. This observation and the protection afforded by spermine against varied swelling agents are indicative of a reaction between the polyamine and a membrane component of mitochondria. (Supported by a grant from the American Cancer Society, Maryland Division.)

6 *Biochemistry*

ADENOSINETRIPHOSPHATASE ACTIVITY OF DESICCATED MITOCHONDRIA. Ralph Penniall. (intr. by J.E. Wilson) Univ. of No. Carolina, School of Med., Chapel Hill, N.C.

Acetone treatment of mitochondria has been shown to solubilize the adenosinetriphosphatase activity. Such powders contain activity that is stimulated by 2,4-dinitrophenol (DNP) and magnesium, and is soluble to sixty minutes centrifugation at 105000xg. Apparent optima for stimulation by DNP and magnesium are at pH 8.5 and 9.0. ATPase is partially activated by cobalt, calcium and magnesium and ferrous ions, but most efficient activation is obtained with manganese. The extracts are unstable, but the stability of the response to magnesium is increased in the presence of magnesium and ATP. The response to magnesium is increased in the presence of reducing agents. The extracts possess no capacity for ATP-P_i^{32} exchange. In the absence of added monovalent cations, the extracts exhibit three apparent peaks of activation in the presence of varying DNP concentrations. (Supported in part by a grant from the National Science Foundation).

7 *Pharmacology*

EFFECTS OF SPERMINE, PUTRESCINE, AND CADAVERINE ON MITOCHONDRIA. Celia W. Tabor. Natl. Insts. of Health, Bethesda, Md.

The OD_{520} mμ of a mitochondrial suspension has been used in many laboratories to follow changes in mitochondrial permeability, a fall in OD_{520} indicating swelling. We have observed that spermine, putrescine or cadaverine inhibit the $-\Delta OD_{520}$ in mitochondria suspended in 0.1 M sucrose-0.01 M Tris HCl pH 7.4 (Lipsett, M., et al., J. Biol. Chem. 234, 2448, 1959). The $-\Delta OD_{520}$ in 12 min. was 0.21 in the absence of spermine, and 0.03 with 0.003 M spermine. In 0.33 M sucrose-0.025 M Tris HCl, pH 7.4 (Hunter, F., et al., J. Biol. Chem. 234,2176, 1959), added succinate results in $-\Delta OD_{520}$ of 0.29 in 34 min.; with $3x10^{-4}$ M spermine, the fall is 0.01. A similar effect was observed with 0.003 M putrescine or cadaverine, but little or no effect with 0.003 M or 0.03 M NaCl, KCl, NH_4Cl, lysine or ornithine. In Hunter's medium at 0°, mitochondria remain dispersed for 16 hrs.; with added amine (0.003 M), agglutination results. With amines, less 260 mμ absorbing material leaks into the medium, suggesting an effect of amines on the permeability of mitochondria similar to that in bacteria (Mager, J.; Herbst, E., and Doctor, B. P.). Experiments on the effects of the polyamines on bacterial systems will also be discussed, with particular reference to the inhibition by spermine of the lysozyme-EDTA induced lysis of E. coli.

8 *Pharmacology*

EFFECT OF TRIETHYLTIN ON MITOCHONDRIAL SWELLING. Kenneth E. Moore* and Theodore M. Brody. Dept. of Pharmacology. University of Michigan, Ann Arbor.

Triethyltin is a potent uncoupler of oxidative phosphorylation and an inhibitor of both Mg and DNP activated ATPases. By measuring photometrically the decrease in the optical density of a dilute suspension of mitochondria in 0.15 M KCl-tris buffer, low concentrations of triethyltin (10^{-7}-10^{-8}M) caused swelling which could be reversed by the addition of ATP and after a lag of 5-10 min. by ADP. Reversal could not be effected by the addition of EDTA. At higher concentrations of triethyl tin (10^{-4}-10^{-6}M) the reversal of swelling by ATP was not observed. The reversal of thyroxine swelling by ATP could be prevented by 10^{-6}M triethyltin. It is postulated that at the higher concentrations triethyltin resembles the actions of azide or p-chloromercuribenzoate in its ability to uncouple oxidative phosphorylation, inhibit ATPases and produce mitochondrial swelling. At concentrations lower than that required to produce uncoupling of oxidative phosphorylation triethyltin may have a direct action on some as yet unknown contractile mechanism of mitochondria. This property of triethyltin may be responsible for some of the toxic actions of this compound. (Supported by NR 303-439, Office of Naval Research).

082. CELLS–Reproduction

9 *Physiology*

REGULATION OF MICRONUCLEAR DIVISION IN STENTOR COERULEUS. E. Guttes, and S. Guttes. (intr. by R. C. Herrin) McArdle Mem. Lab., Univ. of Wis., Madison 6, Wisconsin.

The ciliate Stentor coeruleus has a highly polyploid macronucleus, surrounded by a varying number of micronuclei, which extend longitudinally throughout most of the cell. A ciliary apparatus is present at the anterior end. Cell division is accompanied by mitosis of the micronuclei, and in well fed specimens this occurs every 24-30 hours. Soon after division cells were bisected into anterior and posterior halves, with both parts obtaining approximately equal portions of the macro- and micronuclei. In the posterior halves the micronuclei underwent mitosis about 5-6 hours later (Science 129, 1483, 1959), whereas in the anterior halves their division was delayed. Autoradiographs obtained from specimens incubated with thymidine-H^3 indicate that chromosome duplication occurred in the macronucleus simultaneously with the division of the micronuclei. Since only the anterior half had obtained the ciliary apparatus in this experiment, the difference between both halves with regard to the onset of nuclear division and chromosome duplication may be explained as the consequence of the presence or absence of a site of energy utilization.

2 *Physiology*

THE FERTILIZING CAPACITY OF RABBIT SPERMATOZOA TREATED WITH SONIC BIBRATION. M.C. Chang and D.M. Hunt*. Worcester Fndn. for Exptl. Biology, Shrewsbury, Mass.

Samples of ejaculated rabbit sperm suspended in Ringer-Locke solution in dilution of from 1:20 to 1:500 were exposed to sonic vibration for periods varying from ½min. to 10 minutes (9000 Cycles/sec.). Separation of the sperm head from the tail occurred in 41% and 97% treated for ½ min. and 10 minutes respectively, but a few progressively motile spermatozoa were still present in the latter sample following treatment. Intravaginal insemination of sperm samples treated for ½ to 5 minutes into 5 does resulted in the production of 17 male and 16 female young. It seems that while treated sperm remain motile, there is no impairment of fertilizing capacity, and that sonic vibration produces no differential effect on sperm bearing either the X or Y chromosome. (Supported by grant from the Population Council.)

3 *Biochemistry*

STIMULATION OF CELL DIVISION IN NORMAL RAT LIVER BY A FACTOR IN SERUM FROM HEPATECTOMIZED RATS. M. Zimmerman and E. Celozzi, (intr. by U. J. Lewis.) Merck Institute for Therapeutic Research, Rahway, New Jersey.

Stimulation of mitosis in the liver of normal rats by injection of serum from hepatectomized rats has been reported (Friedrich-Freksa & Zaki, 1954). We have confirmed these results and have examined this phenomenon more closely. The stimulatory factor in serum from hepatectomized rats is non-dialysable, not precipitable by 75% saturated ammonium sulfate and is stable when lyophilized in the presence of other serum proteins. The stimulation of mitosis by injection of such sera is also accompanied by an increase in the incorporation of orotic acid-6-C^{14} into liver DNA. Autoradiography using thymidine-H^3 has demonstrated that virtually all the mitotic figures arising in the stimulated liver are labeled, suggesting that the induced proliferative response represents a complete division cycle and not the division of polyploid or late interphase cells. (Supported by CCNSC, NCI, NIH Contract #SA-43-ph-1886.)

083. CHEMOTHERAPY

4 *Biochemistry*

ABOLITION OF GLYCOLYSIS IN MOUSE TUMOR CELLS BY A COMPLEMENT-DEPENDENT CYTOTOXIC SYSTEM OF NORMAL SERUM. M.W.Woods, M.Landy*, J.G.Michael*, R.-J.Trapani*, B.Achinstein* and M.J.Shear. Natl. Cancer Inst., Bethesda, Md.

Normal human serum, incubated with ascites tumor cells (Sarcoma 37, Ehrlich and Krebs carcinomas) in Krebs-Ringer glucose bicarbonate buffer at 37°C., abolishes their capacity to proliferate in vivo; complement and Ca++ are required for this lethal action (Landy et al., Fed.Proc.,1960). This interaction terminated glycolytic activity of the tumor cells. Loss of capacity to glycolyze and to reduce neotetrazolium, indicative of cell death, was accompanied by blebbing and uptake of trypan blue. After interaction of S37 cells with serum at various temperatures, glycolysis was assayed at 37°C. At 37°C. interaction for 2 min. gave 100% inhibition; at 20°C., 30 min. resulted in 70% inhibition; no inhibition was noted after 60 min. at 4°C. Sera from which Ca++ or any of the recognized components of complement had been removed no longer inhibited. Absorption of serum at 4°C. with tumor cells (S37,Ehrlich and K2 carcinomas, hepatomas, melanoma) removed anti-metabolic activity whereas some normal cells (muscle,heart,lung,kidney,liver) did not. Glycolysis in S37 cells was abolished by sera of 10 animal species. In contrast, glycolysis was increased by sera of 7 other species. Abolition of glycolytic activity afforded a convenient quantitative measure of serum cytotoxicity.

XUM

2 *Biochemistry*

PHYSIOLOGICAL LOSS OF RESISTANCE TO AN ANTIFOLIC ACID DRUG IN A STRAIN OF STREPTOCOCCUS FAECALIS. George H. Hitchings and Robert C. Wood*. Wellcome Research Labs., Tuckahoe, N.Y.

Washed cells of a pyrimethamine-resistant strain of S. faecalis were found to gradually lose their resistance to the drug when they were either stored at 4° for several weeks or incubated overnight with glucose at 37°. Total CF production in the absence of the drug remained constant during the loss of resistance; however, the ratio of free CF to conjugated CF produced from folic acid gradually increased. The production of conjugated CF by these "sensitized cells", and their resistance, could be restored by adding an enriched medium to the reaction system. In contrast, cells of the parent, sensitive strain of S. faecalis rapidly lost their capacity to synthesize both forms of CF and this capacity could not be restored by the addition of the enriched medium.

The CF produced by growing cells of either strain was almost exclusively the conjugated form. This synthesis was inhibited in the wild but not resistant strain by the addition of pyrimethamine. Cell-free extracts of both strains synthesized only free CF from folic acid, and were of equivalent sensitivity to pyrimethamine.

The results suggest that the conjugates may be the more significant metabolites and that the pathways leading to them may be involved in resistance to pyrimethamine.

3 *Biochemistry*

A COMPENSATORY RESPONSE TO A FALSE FEEDBACK INHIBITOR. H. S. Moyed. Dept. of Bacteriology and Immunology, Harvard Med. School, Boston, Mass.

An analog of histidine, 2-thiazole alanine (2-TA), causes a transitory bacteriostasis of Escherichia coli W. The refractory state associated with the resumption of growth is lost by subculture in the absence of 2-TA. The induction of such phenotypic resistance appears to result from the inhibitory effect of 2-TA, mimicking that of histidine, on the action of "compound III" synthetase, an essential enzyme in histidine biosynthesis. Intracellular histidine is consequently depleted, and its repressive effect on the formation of "compound III" synthetase is relieved. Maintenance of the resulting high enzyme levels during growth, like the resistance, are 2-TA dependent. Mutations that decrease the sensitivity of "compound III" synthetase to inhibition by 2-TA correspondingly decrease the stimulatory effect of 2-TA on the formation of the enzyme. Even in concentrations below the bacteriostatic level for the parent organism 2-TA can markedly stimulate the formation of the enzyme. Thus the cell can compensate for the inhibitory effect of low doses of an antimetabolite which otherwise would be strongly inhibitory. This compensatory response is possible because 2-TA mimics histidine as a feedback inhibitor but not as a repressor of "compound III" synthetase.

4 *Pharmacology*

METABOLISM OF CHLORGUANIDE IN MAN. Carl C. Smith and Jean Ihrig*, Christ Hospital Institute of Medical Research, Cincinnati, Ohio.

The metabolic disposition of the anti-malarial drug, chlorguanide (CG), was studied in four human subjects. Following single oral doses of 300 mg., urinary excretion was essentially complete in two days; 56 to 66% of the dose administered was recovered as p-chloroaniline (PCA) containing metabolites. Countercurrent distribution studies indicated that the major PCA-containing metabolites were CG, chlorguanide triazine (CGT), and p-chlorophenylbiguanide (PBG). CGT excretion was determined microbiologically utilizing its unique antifolic acid activity. PBG was condensed with acetone to form CGT, then measured microbiologically along with preformed CGT. The difference between this total value and that for preformed CGT was ascribed to PBG. CG excretion can then be obtained by difference between total PCA and PCA equivalents of CGT and PBG. CG accounted for 46-76%, CGT for 17-43%, and PBG for 5-11% of the total urinary metabolites. Comparison of these results with previous studies in monkeys indicated that the metabolic patterns in these two primates are qualitatively similar but that monkeys convert a larger percentage of CG to PBG. (Supported by grant E-824, National Institutes of Health.)

5 *Pharmacology*

METABOLISM OF SULFINPYRAZONE IN MAN. Peter G. Dayton*, Leonardo E. Sicam*, Miguel Landrau* and J. J. Burns. Natl. Heart Inst., Bethesda, Md. and N.Y.U. Res. Serv. Goldwater Mem. Hosp., N.Y.

Previous studies showed that a thio-ether analogue of phenylbutazone (C-25671) was metabolized in man to the corresponding sulfoxide (J. Pharm. and Exptl. Thera. 119, 418, 1957). In view of the usefulness of sulfinpyrazone as a uricosuric agent in the treatment of gout, the further metabolism of the drug was investigated. After administration of sulfinpyrazone to human subjects a metabolite was isolated from urine which has a para-hydroxy group on one of the benzene rings attached directly to the pyrazolone nucleus. This metabolite was identified by comparison with a synthetic reference compound employing the following criteria: infrared and ultraviolet absorption spectra, melting points and partition coefficients. About 5% of an 800 mg intravenous dose of sulfinpyrazone was recovered in urine as this metabolite. The biological half-life in man of the metabolite was 1 hour compared to 3 hours for sulfinpyrazone. The sulfone analogue of sulfinpyrazone appears not to be an important metabolic product of the drug.

6 *Pharmacology*

EFFECT OF ORGANIC ACIDS ON OXIDATIVE METABOLISM OF ESCHERICHIA COLI. P. Draskoczy* and N. Weiner. Dept. of Pharmacology, Harvard Medical School, Boston, Mass.

The effect of mandelic acid (MA), hippuric acid (HA), acetic acid (AA), and lactic acid (LA) on the oxidative metabolism of E. Coli has been evaluated at different concentrations of acid and different values of pH. All produce an immediate inhibitory effect which is directly related to the concentration of unionized molecules, and only indirectly related to pH and the total concentration of acid. The concentration of unionized acid required to inhibit depends on the acid used. MA and HA are approximately equally effective in terms of concentration of unionized molecules. LA is about 1/10 as effective, and AA is slightly less effective than LA. The concentrations of unionized molecules which produce 40-50% inhibition of oxidative metabolism are in the same range as those required to inhibit bacterial growth (Kass et al., Unpub.). Studies with disrupted bacterial fractions indicate the cell wall may play a role in the prevention of the inhibitory effect of organic acids at low concentrations of unionized acid. (Supported by USPHS grants H-2205 and H-3674.)

7 *Pharmacology*

PROTEIN BINDING OF OCTOIODOFLUORESCEIN-I^{131}. Edward C. Tocus* and George T. Okita. Argonne Hosp. and Dept. of Pharmacology, Univ. of Chicago, Chicago.

Octoiodofluorescein-I^{131} (OIF-I^{131}) has been shown to localize in brain tumor tissue. Some factors concerning the mechanism of localization were determined in this study using the techniques of electrophoresis, autoradiography and microphotography. Electrophoretic studies gave the migration patterns of free OIF-I^{131} and free I^{131}. Proteins obtained from mouse serum showed OIF-I^{131} bound to the albumin fraction and a small amount of free I^{-131}. Normal mouse brain proteins contained no measurable OIF-I^{131}. Proteins from a mouse neuroblastoma contained OIF-I^{131} in the albumin and globulin fractions. Perfused normal mouse brain was found by autoradiography to contain little I^{131} except in areas unprotected by the blood brain barrier. Proteins from human brain tumors when separated by electrophoresis showed OIF-I^{131} bound to the albumin and globulin fractions. In addition, microphotography demonstrated that sections taken from a human brain tumor showed tumor cells stained in vivo with OIF.

8 *Pathology*

ON THE PREVENTION OF TOXICITY OF NITROGEN MUSTARD. L. P. White (intr. by Sidney Farber)Children's Cancer Res. Fndn., Boston, Mass.

Experiments to be reported have demonstrated that the toxic effects of nitrogen mustard in mice can largely be prevented by injecting the HN2 at a pH of 2.0.

It has previously been shown that saline homogenates of spleen plus nitrogen mustard have greater toxicity than the same amount of HN2 alone. The pH of homogenate-HN2 mixtures was always higher than that of HN2 alone. When pH of HN2 was raised to 7.0 with bicarbonate, toxicity was increased. Accordingly, mixtures of HN2 in varying doses from 2.25-20.0 mg/kg were made up at pH values ranging from 2.0-11.0, Upon intraperitoneal injection of these materials in Swiss mice, lethality of a dose was found to depend directly upon the pH of the solution, increasing with rising pH. At lower doses (3.0-4.5 mg/kg) no deaths were seen from HN2 at pH 2.0. At pH8.0 the same doses were uniformly lethal. At intermediate pH (4.0-6.0) intermediate numbers of deaths occurred. At doses of HN2 above 6.0 mg/kg survival was seen only at pH 2.0. Regardless of pH no mouse survived a single dose of more than 10.0 mg/kg, but when doses were fractionated into 5 daily doses, this total amount could be given at pH 2.0, not at higher pH. Intravenous administration of HN2 showed even more striking pH dependent differences, with rapid deaths from pH8.0 material, and no deaths at pH 2.0, both using HN2 4.0 mg/kg.

084. CHEMOTHERAPY–Antibiotics

Physiology

PREVENTION BY SODIUM SALTS OF STRESS-ELICITED CARDIAC NECROSIS AND NEPHROCALCINOSIS, IN NEOMYCIN-TREATED RATS. S. Repaud (intr. by E. Robillard) Institut de Médecine et de Chirurgie expérimentales, Univ. de Montréal, Canada.

When various stressor agents, such as forced immobilisation quadriplegia, spinal cord transection, intestinal lesions or morphine sulfate, are applied to 200gm female Sprague Dawley rats receiving 60mg/Kg of neomycin sulfate, subcutaneously, twice daily, marked toxic effects are elicited in a high percentage of the animals, while the controls receiving neomycin alone, at the same dosage, do not show any significant adverse effects. The lesions consist of large patches of cardiac necrosis located mainly in the right ventricle, and of massive calcifications of the kidney with desquamation of the proximal tubular epithelium and hyaline casts. A similar toxic pattern results when neomycin is given alone at a very high dosage (150mg/Kg twice daily). In both cases, concomitant oral administration of sodium chloride or bicarbonate (2 mM twice daily), prevents almost completely the mortality and the appearance of the lesions. It is concluded that these toxic neomycin reactions are largely dependent upon conditioning by nonspecific stress and that sodium salts could be of valuable help in preventing them. Supp. by Natl. Res. Council Canada; Gr. No. H-3688(C2), U.S.P.H. Natl. Heart Inst; Pfeiffer Res. Found.

2 *Biochemistry*

THE CONVERSION OF D-GLUCOSE TO 5-KETO-6-DEOXY-D-ARABOHEXOSE. A. D. Elbein*, R. L. Mann, H. E. Renis*, W. M. Stark*, Henry Koffler, and H. R. Garner*. Dept. of Biol. Sci., Purdue University, Lafayette, Ind., and the Lilly Research Labs., Indianapolis, Indiana.

The antibiotic, hygromycin A, has been shown to contain 5-keto-6-deoxy-D-arabohexose. After growing the organism in a synthetic medium for 72 hrs., labeled glucose (1-C^{14}, 2-C^{14}, or 6-C^{14}) was added; growth continued for 24 hrs. The antibiotic was isolated, the keto group reduced with $NaBH_4$, and the sugar isolated as L-fucose diethylmercaptal. The latter was converted to L-fucose, and its benzimidazole derivative prepared. The fucobenzimidazole was degraded and incorporation of C^{14} into various carbons was studied. When the organism was grown in the presence of 1-C^{14}- or 2-C^{14}-D-glucose, 87-90% of the total activity in the sugar was found in carbons 1 or 2. When the organism was grown on 6-C^{14}-D-glucose, 95% of the activity was found in carbon 6. These observations suggest that the carbon chain of D-glucose is converted to that of 5-keto-6-deoxy-D-arabohexose without skeletal rearrangement.

3 *Pharmacology*

RELATION OF PLASMA LEVELS OF CYCLOSERINE TO ORAL DOSAGE AND CLINICAL RESPONSE IN PULMONARY TUBERCULOSIS. Israel G. Epstein*, K. G. S. Nair*, Michael G. Mulinos, Emanuel Horiuchi*, and Benjamin Encarnacion*. N.Y. Medical College, Metropolitan Medical Center, and Commercial Solvents Corporation, New York, N.Y.

Sixteen adult patients with moderately to far advanced previously untreated pulmonary tuberculosis were treated with cycloserine (CS). The dose of 0.5 Gm./day was increased stepwise until the plasma level of CS reached 30 to 50 mcg./ml., where it was maintained. Body weight gain averaged 13 lbs.; fever was abolished within 32 weeks of treatment. Clinical, X-ray, and sputum clearance improvement was marked in 75% of cases. Cycloserine plasma levels averaged 14.6 mcg./ml. in 13 subjects on 0.5 Gm./day; 27.5 mcg./ml. in 11 on 1.0 Gm./day; 39.2 mcg./ml. in 11 on 1.5 Gm./day, and 42.3 mcg./ml. in 10 on 2.0 Gm./day. The dose levels of CS (0.5-2.0 Gm./day) represented from 7.4 to 34.5 mg./Kg. The CS plasma levels showed little correlation with oral dose among these 16 subjects collectively. However, individually the data show excellent plasma level to oral dose correlation, the levels in each subject being maintained with good consistency during this study. Three subjects had transitory tremors, one occurring after the addition of INH. In no case was it necessary to discontinue therapy because of toxicity.

4 *Pharmacology*

FURTHER PHARMACOLOGICAL STUDIES ON PROPIONYL ERYTHROMYCIN ESTER LAURYL SULFATE. Cheng-Chun Lee, Robert C. Anderson and Verlin C. Stephens*. Lilly Research Labs., Indianapolis 6, Indiana.

The pharmacology and toxicology of erythromycin, propionyl erythromycin and its lauryl sulfate salt (PELS) have been reported from these laboratories. This report concerns the blood and tissue partition of the antibiotic following oral administration of PELS and the distribution of radioactivity after oral administration of S^{35}-labelled PELS. In the rats, about 40% of the total blood erythromycin was loosely bound to or adsorbed on the cells. In the human subject, the blood cells contained 20% of the total blood erythromycin. Both erythromycin and propionyl erythromycin were present in the blood and various tissues. Radio-isotope studies with PELS-S^{35} indicated that S^{35} was absorbed through the gastrointestinal tract of the rat. Most of the absorbed radioactivity was eliminated in the urine and bile. In the urine, only 20% of the radioactivity was found in the form of inorganic sulfate and lauryl sulfate. Radio- and bio-autographic studies indicated that radioactivity was not associated with biological activity.

5 *Pharmacology*

THE DISTRIBUTION OF TETRACYCLINES IN THE DOG. Kurt W. Kohn,* James Gaskins,* and David P. Rall, National Cancer Institute, Bethesda, Maryland

Tetracycline (TC) and demethylchlortetracycline (DMCT), 5.0 mg/kg, were given i.v. to dogs anesthetized with pentobarbital or chloralose. When constant plasma levels were required, drug infusions were added. Drug concentrations were determined by fluorometry of the tetracycline-calcium-barbital complexes extracted into ethyl acetate, as described previously (ACS Abstracts, April, 1959, 69C). Binding to plasma proteins (equilibrium dialysis at 38°C) was TC 35-56% (average 42%, 7 dogs) and DMCT 48-70% (average 57%, 10 dogs). The apparent volumes of distribution (in terms of total plasma concentrations) were TC (3 dogs): 0.58-0.64 and DMCT (5 dogs): 1.1-1.4 liters/kg. Both drugs entered skeletal muscle. DMCT attained considerably higher concentrations in muscle than in plasma during maintenance of relatively constant plasma levels. The concentration ratios, muscle (wet weight) to plasma (total), were TC 1.0-1.5 (average 1.2, 4 dogs) and DMCT 1.6-2.5 (average 2.1, 8 dogs). In terms of free plasma concentrations, these ratios are approximately 2.2 for TC and 5.3 for DMCT. Erythrocyte-to-plasma concentration ratios were 0.7-1.0 for TC (8 dogs) and 0.6-1.2 for DMCT (6 dogs). Spinal fluid levels after 4-8 hours of equilibration were only 14-19% (TC, 5 dogs) and 17-31% (DMCT, 3 dogs) of the free drug concentrations in plasma.

XUM

085. CHEMOTHERAPY–Cytotoxic Drugs

Pharmacology

PHARMACOLOGICAL STUDY OF AMMONIATED ETHYLENE MALEIC ANHYDRIDE POLYMER, M. W. 20,000-30,000 (AEMA 20-30). R. Mihich, C. L. Simpson*, W. Regelson* and A. I. Malberg*. Roswell Park Memorial Institute, Buffalo, N. Y.

Significant inhibition of 6 experimental tumors was found in rodents treated with AEMA 20-30, (W. Regelson et al. Proc. Am. Ass. Cancer Res. 3, 55, 1959). This study was undertaken prior to possible clinical testing of this drug. Severe local toxicity was observed in rodents. In dogs AEMA 20-30 was lethal to one half of the animals at 25 mg/Kg/day, i.v. (5 doses per week for 2 weeks). Loss of weight and bloody diarrhea were noted. Very striking hematological alterations were: marked normocytic anemia (30%-60% reduction of RBC) with a significant increase of circulating nucleated red cells (up to 21 per 100 WBC) without a corresponding increase of reticulocytes; peripheral WBC increase up to 140,000 per cu.mm (immature and atypical cells up to 20%). The same type of hematological derangement was seen in rats treated with the drug (100 mg/Kg/day, i.p., for 5 days). In dogs blood biochemical evidence of altered liver function was obtained. Microscopically there was proliferation of the RES with secondary necrosis of liver parenchyma. Intravascular thrombosis was evident mainly in the lungs. A possible relationship between the diversified effects observed and the alterations of the RES will be discussed.

2 *Pharmacology*

THE FLUORIMETRIC ESTIMATION OF URACIL NITROGEN MUSTARD AND ITS BIOLOGICAL DISPOSITION IN THE DOG. L.B. Mellett and L.A. Woods. Dept. of Pharmacology, Univ. of Michigan Med. School, Ann Arbor.

Information on plasma levels and urinary excretion of 5-bis (2-chloroethyl)amino-uracil (uracil nitrogen mustard, Upjohn U-3844) after intravenous and oral administration has not been available up to the present. The method of estimation consists in coupling the alkylating agent with p-phenylphenol to produce a fluorescent product which is then quantitatively determined in the Aminco-Bowman Spectrophotofluorimeter at an activation wave length of 285 mμ and a fluorescent wave length of 340 mμ. Female mongrel dogs receiving 1.0 mg/kg intravenously displayed plasma levels of 2.1 to 2.2 μg/ml in the initial sample (ca. 3 minutes). These levels declined rapidly so that within one to two hours the readings were equivalent to control levels. Urinary recovery of the drug in these animals was less than 0.9%. Female dogs receiving 2.0 mg/kg orally showed low initial plasma levels which attained a maximum of 1.1 to 1.2 μg/ml at 20 minutes and returned to control levels within 1½ hours. Urinary recovery in these animals was less than 0.2%. From these data it is concluded that the compound is absorbed quickly but not completely after oral administration in dogs. (Supported by USPHS Grant CY 2992).

3 *Pathology*

CORRELATION BETWEEN CYTOTOXICITY IN HUMAN CELL CULTURE AND "IN VIVO" ANTICANCER ACTIVITY OF PURINE ANALOGS. G.J.Dixon,* H.E.Skipper, and F.M. Schabel, Jr., Southern Research Institute, Birmingham, Alabama.

Following the publications of Eagle and Foley, 89 purine analogs have been studied with regard to cytotoxicity to human cancer cells (KB) in tissue culture (T.C.) and Adenocarcinoma 755, an experimental tumor which appears to parallel certain human leukemic response to purine antagonists. Of the 48 purines which inhibited Ca755, 88% were cytotoxic in T.C. at 100 mcg/ml or less. However, 20 out of 41 (49%) of the Ca755 negatives also inhibited T.C. at 100 mcg/ml or less. Sixteen of these so-called "false positives" were adenine derivatives, some of which might be active in some other "in vivo" tumor system. Among the Ca755-positive purines there appeared to be no obvious correlation between "in vivo" therapeutic index and degree of T.C. cytotoxicity. There also appeared to be almost as good a correlation between animal toxicity and human cancer cell cytotoxicity as was noted for antitumor activity vs. T.C. cytotoxicity. (Supported by the National Institutes of Health.)

087. CONNECTIVE TISSUE

Physiology

A BASIS FOR COLLAGEN-APATITE INTERACTION. H. Elden*, Howard Hughes Medical Institute, Miami, Florida, and D. S. Howell, University of Miami, Coral Gables, Florida.

Hydroxyapatite-crystal-growth in osteoid matrix appears to begin in apposition to collagen fibers. It is not known how these crystals interact with the surface of the protein. Studies on a model system, reported herein, suggest a possible mode of interaction. A suspension of hydroxyapatite was mixed at pH 7 with certain small molecules having functional groups found in collagen. Light scattered by these mixtures was measured and compared with summation of intensity of light scattered by components; the ratio, (meas. intens./calc. intens.), provided an index of interaction (n). Glycine, tyrosine, arginine, and histidine failed to interact whereas, lysine, aspartic and glutamic acids, serine, threonine, and hydroxyproline did interact. For -OH and $-NH_2$ groups n increased (0.7 to 1.8) in the series methyl, ethyl, iso-propyl, n-propyl alcohol; methyl and ethylamine. A smooth continuous function resulted when n's were plotted against the "a" terms of the Van der Waals gas equation. An interaction is postulated between negative centers in these molecules and the positive surface charges of the crystal; similar interactions might be a basis for attachment of hydroxyapatite to collagen via side chain polar groups.

2 *Physiology*

INTERACTIONS OF AGE AND FORMALDEHYDE ON THE MECHANICAL PROPERTIES OF RAT TAIL TENDON. John D. Spikes. Dept. of Experimental Biology, Univ. of Utah, Salt Lake City 12, Utah.

The load-strain (LS) and stress-relaxation (SR) behavior of wet rat tail tendon was studied as a function of animal age, formaldehyde (F) concentration and pH. LS curves are not linear but show a marked "toe" region. Both LS and SR are affected by the rate and extent of straining employed. F altered the final slope of the LS curve only slightly, but progressively decreased the "toe" region and the rate of SR over the range 0.001-0.1 M. F produced a pH-dependent contraction of the tendon. No contraction occurred below pH 6.5. The force produced by contraction increased rapidly from pH 6.5 to 7.0 and then remained constant up to pH 11.0 Other than this, pH had relatively little effect on tendon mechanical properties, except above pH 11 and below pH 5 where the tendon was unstable. Tendon from animals younger than 2-2.5 months was difficult to study because of its fragility; however, measurements on F-treated tendon from 3-4 week old rats could be made. The LS curve increased slope with increasing age of animal, while the rate of SR decreased rapidly from 2 months to 4 months and then remained approximately constant to 28 months. F decreased the rate of SR much more in tendon from young animals than from older animals. (Supported by grant H-3039 from USPHS-NIH).

3 *Biochemistry*

ISOLATION OF A GLYCOPROTEIN FROM RAT GRANULOMAS. A. F. Fishkin and G. S. Berenson (intr. by R. A. Coulson.) Louisiana State University School of Medicine, New Orleans.

The inflammatory reaction is a fundamental protective mechanism of connective tissue. Experimental models, i. e. sirturpentine granulomas produced in rats, aid in study of the intricate biochemistry of inflammation. In these experiments a glycoprotein was isolated from seven-day-old granulation tissue by a method which included water extraction, dialysis, concentration at 80° with elimination of heat-coagulable protein and precipitation with alcohol. Purification by zone electrophoresis on starch with a borate-sulfate buffer, pH 8.6 and μ 0.1, permitted isolation of a homogenous compound with a sedimentation constant of 2.2 S. The following analyses, per cent of dry weight, were obtained: hexosamine-4.7, sialic acid-12.9, methylpentose-0.9, polypeptide-56.0, nitrogen-12.7, and phosphorus-0.1. A content of 2.8% tyrosine and 3.7% tryptophan was estimated from spectrophotometric data. Paper chromatographic studies indicated the presence of 14 to 16 amino acids, galactosamine and mannosamine and/or glucosamine. The isolation of a glycoprotein and four acid mucopolysaccharides in earlier studies of granulomas, and collagen by others provides further evidence of the complexity of composition and of the active metabolic properties of inflammatory tissue.

4 *Biochemistry*

BINDING OF ANIONS TO CONNECTIVE TISSUE SURFACES. Norman R. Joseph, Milton B. Engel* and Daniel M. Laskin.* University of Illinois Professional Colleges, Chicago, Illinois.

By electrometric measurements of colloidal charge, titration curves of connective tissue of rats and rabbits were determined in vivo between pH 7.4 and 2.2 (Arch. Biochem. and Biophys., 84: 224, 1959) Tissues studied included dermis, tendon and cartilage. The effects of a number of anions on colloidal charge were studied at various pH's. The anions included drugs (salicylic acid and several congeners), dyes (alizarin and derivatives), metabolic inhibitors (dinitrophenol, iodoacetate) and metabolites (ascorbic acid, lactate and bicarbonate ions). At physiological pH anions of these various kinds are bound to the colloidal surface with simultaneous uptake of hydrogen ions and lowering of negative colloidal charge. Displacements of the titration curves resemble those caused by various anions at the surface of insoluble fibrous proteins. The effects are due to thermolabile anion-cation exchange reactions. The free energies of exchange are low, leading to very high labilities of the reactions.

5 *Biochemistry*

EXTRACTION OF GLYCOPEPTIDES FROM GRANULATION TISSUE. Barbara Robert* and Ladislas Robert* (intr. by O. Bergeim), Dept. of Biol. Chem., Med. Fac., Paris, Dept. of Biol. Chem., Univ. of Ill., Coll. of Med., Chicago, Ill.

Carrageenin granuloma extracts from guinea pigs were separated in dialysable, 75% alcohol-soluble and insoluble fractions. The dialysate of the saline washing of the tissue is rich in free amino acids and small peptides. High voltage electrophoresis shows aspartic acid, alanin, several other neutral amino acids, lysine, glucosamine and several peptides. Doca-treated animals have much more free amino acids and peptides than control or cortisone treated animals. The alcoholic supernate of a borate extract was separated in four fractions, Amberlit-IR 120-NH_4OH 1N, Dowex-1-Acetic Acid .1N and HCl .25N and unabsorbed effluent. 62% of total hexose (1 mg/gr fresh tissue) was recovered in these fractions as indicated in the same order, 9%, 11.3%, 4.7% and 75% respectively. All fractions contain unreactive sugar, liberated by acid hydrolysis (glucosamine, galactose, glucose, mannose, fucose) as well as several amino acids. The HCl-eluate has two more spots, a pentose and another migrating with the Rf of rhamnose. These findings may be related to the high glycoprotein level of carrageenin-treated animals.

6 *Biochemistry*

THE SYNTHESIS AND TURNOVER OF COLLAGEN AND ELASTIN IN TISSUES OF RAT. K.-Y. T. Kao, D. M. Hilker and T. H. McGavack (intr. by Leon Swell). Geriatrics Res. Lab., V. A. Center, Martinsburg, W. Va. & George Washington Univ. School of Med., Washington, D. C.

Female rats, 5 wks. old, were each injected with 6.25 μc. of uniformly-labelled C^{14}-lysine. In groups of 6, the rats were sacrificed at different intervals after injection. Skin, tendon, aorta and uterus were fractionated into nonsclero-protein (NSP), alkali-soluble collagen (SC), insoluble collagen (IC) and elastin (E). In the aorta, specific activity (SA-cpm/mg of protein) of the NSP and SC reached its maximum at 24 hrs. (220 & 140) and then decreased with a $t\frac{1}{2}$ of 9.4 and 10.0 days. The IC and E were still at their max. SA 30 days after injection (36.0 & 22.5). In tendon, the SA of NSP and SC reached its max. at 72 hrs. (170 and 81) with a $t\frac{1}{2}$ of 12.2 and 15.0 days. The IC was still at its max. SA 30 days after injection (29). In the skin, the SA of NSP and SC reached its max. in 24 hrs. (222 & 165) with a $t\frac{1}{2}$ of 9.3 and 9.8 days. The SA of IC reached its max. at 3 days and maintained that value for 17 days. The $t\frac{1}{2}$ was reached 8.3 days later. In the uterus, the SA of NSP and SC reached its max. in 4 hrs. (390 & 260) with a $t\frac{1}{2}$ of 6.2 and 6.7 days. The SA of IC reached a max. on the tenth day and stayed at that value to the 20th day, with a $t\frac{1}{2}$ reached 6.7 days thereafter.

7 *Pharmacology*

HYDROXYPROLINE AND COLLAGEN TURNOVER. D. Prockop*, S. Lindstedt*, and S. Udenfriend. Natl. Heart Inst., Bethesda, Md.

Studies by Neuberger and Slack (Biochem. J. 53: 47, 1953) with glycine-C^{14} indicated very slow turnover rates for collagen. Recently we have reinvestigated collagen turnover with a new technique (in prep.) for measuring the specific activity of hydroxyproline (HOPr). Rats were given 25 to 50 μcuries of proline-C^{14} and the labeling of HOPr was followed for 3 months. In young rats the fall of HOPr specific activity in urine approximated a three component curve with half-lives of 2 days, 15 to 20 days, and 100 to 300 days. In mature rats the fall of urinary specific activity showed only two components and these had half-lives of 2 days and over 300 days. These findings suggest that some pools of collagen or collagen precursors turn-over at rapid rates. Preliminary measurements of skin collagen fractions suggest that the initial fall of urinary specific activity reflects the turnover of soluble forms of collagen. Differences between young and old rats are consistent with previous reports that old animals have less soluble collagen, and they raise the possibility that collagen must be converted to a soluble form before degradation.

8 *Pharmacology*

THE STRUCTURE OF ELASTIN AS ELUCIDATED BY ELASTASE. Frank S. LaBella (intr. by Peter E. Dresel). Univ. of Manitoba Faculty of Medicine, Winnipeg, Canada.

Elastin powder was prepared from beef ligamentum nuchae by alternate fat extraction, refluxing in N/10 NaOH, and grinding in a hammer mill. The insoluble protein thus purified still retains, besides pseudoplasmalogen and a phosphorus component previously reported, an additional Schiff-positive substance, a fluorescent moiety, and a yellow pigment, all of which are firmly bound. Incubation of the purified fibrous protein with a highly active preparation of elastase releases trichloroacetic acid (TCA)-soluble protein and TCA-insoluble protein. The TCA-insoluble protein is further attacked by the enzyme, the non-dialyzable products being precipitated only by increasing concentrations of TCA as digestion progresses; proteolysis is accompanied by loss of Schiff positivity and by release of some dialyzable nitrogen. Soluble protein is released from the elastic fiber exponentially suggesting that stabilizing bonds are gradually weakened. The fluorescent component and the yellow pigment remain non-dialyzable throughout proteolysis and are apparently identical. Chromatographic separation of acid hydrolysates of elastin powder indicates the presence of a family of compounds related to pyridoxine and/or pteridine. (Supported by a grant from the American Heart Association).

9 *Pathology*

EXPERIMENTAL AMYLOIDOSIS INDUCED BY TURPENTINE ABSCESSES. Paolo Mombelloni*, Conrad L. Pirani and Hubert R. Catchpole. Department of Pathology, University of Illinois College of Medicine, Chicago, Illinois.

Sterile subcutaneous abscesses were induced in mice by injecting 0.1 ml. of turpentine twice weekly for periods of up to two months. Groups of injected animals and their controls were killed by exsanguination at 15, 30, 45 and 60 days. Blood sera were analyzed for proteins and mucoproteins. Autopsies and histologic studies were carried out in all animals. Amyloid was present in the spleen of 20, 40, 75 and 100 per cent of the mice killed respectively at 15, 30, 45 and 60 days. Less amyloid was present in liver and kidneys. The presence of amyloid was associated with a marked reticulo-endothelial hyperplasia in both spleen and liver. In the injected animals with amyloidosis, a mild increase in total serum protein was associated with a decrease of albumin and an elevation of the globulins in the alpha2 and particularly in the beta fractions. The protein-bound hexoses progressively increased to a maximum of 50 per cent above the normal levels. This was associated with a marked increase in the alpha2 and beta fractions in PAS stained electrophoretic strips. The serum protein changes were similar but less marked in mice which failed to develop amyloidosis. (Supported by a grant from the USPHS, Grant A 968C3.)

XUM

10 **Pathology**

EFFECT OF HYDROCORTISONE, ESTRADIOL AND MAST CELL DEPLETION ON CARRAGEENIN GRANULOMA. J.A. Paar* and Edwin R. Fisher. Univ. of Pgh. and VA Hospital, Pgh., Pa.

Subcutaneous injection of carrageenin, a sulphated polygalactose of Irish moss, has been observed to produce a collagen-rich granuloma subsequently replaced by adult adipose tissue. This process has been investigated by appropriate histochemical technics in guinea pigs 1)stock laboratory diet (42 mg./100Gm. ascorbic acid;2) stock diet and parenteral ascorbic acid (25 mg./day);3) hydrocortisone (5 mg./day;4) estradiol (.0005 mg./day). Animals were sacrificed 1-50 days following carrageenin. Untreated animals and those receiving ascorbic acid disclosed maximum collagen formation 15 days after injection which was preceded by an increase in reticulin, fibroblastic and histiocytic activity. No intercellular acid mucopolysaccharide was evident. Degeneration of collagen was evidenced by clumping of collagen masses exhibiting sudanophilia, argyrophilia and AMP. Altered collagen appeared engulfed and phagocytized by macrophages rich in lipase and ingested carrageenin. Vacuolation,sudanophilia and presence of adult adipose tissue at 45-50 days suggested an origin of the latter from such cells. A quantitative diminution of all phases of this reaction was observed in animals receiving hydrocortisone whereas the converse effect was noted with estradiol. Mast cell depletion in the rat produced by compound 48/80 failed to alter the carrageenin granuloma in this species.

11 **Pathology**

ELASTASE PRODUCTION BY ACTINOMYCES. A.J. Sbarra*, R.F. Gilfillan*, & W.A. Bardawil, St. Margaret's Hosp.-Path. & Medical Res. Labs., & Tufts Univ. School of Med., Boston, Mass.

It is tempting to implicate elastase as a contributing factor in the pathogenesis of Temporal Arteritis, arteriosclerosis, and other vascular diseases. Resected temporal arteries from two different patients afflicted with Temporal Arteritis have, as a result of bacteriological examination, revealed the presence of ACTINOMYCES sp. This finding, known for the past thirty years, has received little attention, since their presence was considered to be a result of contamination and not an association with etiology. Recently we have been able to isolate from soil at least five different micro-organisms that produce elastase. Two of these are members of the family ACTINOMYCETACEAE. The enzyme, elastase was extracted from a 3-5 day culture of ACTINOMYCES sp. grown on either an elastin containing medium or Fields Tryptic Digest Broth at room temperature. Preliminary experiments indicate that the enzyme closely mimics the bovine elastase. For example: it is inhibited by human serum, it shows a similar pH optimum, and end product analysis reveals a similar pattern,in so far as amino acids are concerned. An evaluation of the pathological changes induced by this organism will be discussed (Supported by a Grant from USPH-B1962 and A2168

12 **Pathology**

AN ELASTIN ELASTASE SERUM INHIBITOR SYSTEM: END PRODUCT ANALYSIS. R.F. Gilfillan*, A.J. Sbarra*, and W. A. Bardawil, St. Margaret's Hosp., & Tufts Univ. School of Med., Boston, Mass.

The system has been studied with regard to kinetics and end-product formation in the presence and absence of serum inhibitor(s). Collagen-free and relatively lipid-free elastin was prepared from ligamentum nuchae, and a purified pancreatic elastase, from hog trypsin 1-300. Analysis of reaction mixtures, using paper chromatography, indicate the liberation of six free amino ninhydrin-positive substances, identified in decreasing concentration as alanine, glycine leucine, valine, and proline. Measurement of products formed is more directly proportional to substrate dissolved over a longer range of linearity than nephelometry. Serum obtained from a) normal females, b) placental cord blood, c) third trimester pregnancy, and d) temperal arteritis produced in optimal equiv. conc. 52%, 9%, 91% and 40% inhibition respectively when measured nephelometrically. Analysis of reaction mixtures indicates quant. inhibition of products formed and may provide a sensitive inhibitor assay in the study of pathogenesis of vascular diseases. (Supported by Grants from USPH B1962 and A2168)

13 **Pathology**

MECHANICAL STRESS AND CONNECTIVE TISSUE DYNAMICS. Jackson J. Clemmons. Institute of Pathology, Western Reserve University, Cleveland, Ohio.

There are few reports concerning the in vivo reaction of fibrous connective tissue to mechanical stress. Biochemical studies indicate that this tissue is metabolically relatively inert, but it is not known whether this represents only the static phase for tissue capable of becoming dynamic when adjusting to altered stress. A procedure was devised whereby mechanical stress of known magnitude and frequency can be applied to the skin of rats. The tension applied was equal to that present in the human aortic wall. This tension produced atrophy of the panniculus carnosus and an apparent increase in density of dermal collagen. Aminoacetyl nitrile (AAN) was administered to a second group of animals to interfere with collagen formation, but in the absence of stress no alteration in the dermis was observed. In a third group receiving mechanical stress and AAN there occurred extensive degeneration and necrosis of muscle, and connective tissue. It is concluded that stress can change fibrous connective tissue from a static to a dynamic state.

14 **Pathology**

AMINO ACID COMPOSITION OF AORTIC ELASTIN ISOLATED BY THE USE OF FORMIC ACID. John P. Ayer and Laila Galins.* Cancer Research Institute, New England Deaconess Hospital, Boston, Mass.

Using ion-exchange column chromatography the quantitative estimation of the amino acids in the elastin of dog and calf aorta purified with concentrated formic acid has been obtained. The results for calf elastin are similar to published results for bovine elastic tissue isolated by different methods. The amino acid composition of dog elastin differs quantitatively from that of calf elastin, particularly in the greater amounts of histidine, threonine, leucine and tyrosine. The latter composition has been confirmed by studies using ultraviolet absorption. That no significant release of free amino acids from the tissue by the formic acid was proved by paper chromatography using unhydrolyzed elastin (Supported by the Atomic Energy Commission).

088. CONNECTIVE TISSUE–Disorders

1 **Pathology**

EFFECTS OF A TOXIC CELLULAR COMPONENT OF GROUP A STREPTOCOCCI ON THE ARTICULAR TISSUE OF RABBITS. William J. Cromartie, John H. Schwab* and John T. Langley.* Depts. of Bacteriology and Medicine, Univ. of N.C. School of Med., Chapel Hill, N.C.

A cellular component of group A streptococci which produces a prolonged toxic effect on connective tissue following a single injection has been isolated and characterized as a macromolecular complex of a polysaccharide having the serological and chemical characteristics of Group-specific C substance (Schwab,Cromartie,Roberson, J. Exper. Med. 109:43, 1959). The effects of the direct injection of 0.2 ml of an extract containing this toxic substance into the left knee joint of each of 30 rabbits was studied. A comparable extract of group D streptococci was injected into the opposite joint. A marked hyperplastic reaction of the synovial membrane was observed to persist for at least 36 days after injection of the extract of group A streptococci whereas the acute reaction observed after injection of the group D material subsided completely by the 16th day. The chronic reaction observed after injection of the group A material was characterized by hyperplasia of the synovial lining cells, diffuse infiltration of the villi by macrophages, and giant cells and focal collection of lymphocytes. These observations combined with other studies suggest that this type of toxic material may be of importance in the pathogenesis of certain diseases of connective tissue including rheumatoid arthritis.

2 *Pathology*

INFLUENCE OF β-AMINOPROPIONITRILE FEEDING ON COLLAGEN SYNTHESIS IN RAT AORTAS. Joseph J. Lalich, Douglas Angevine* and Herman Wirka*. Univ. of Wisconsin Med. School, Madison, Wis.

In β-aminopropionitrile (BAPN) fed rats which did not die of angiorrhexis, weakening of the media predisposed to aneurysmal dilatation and fibrosis of the aorta. Chemical analyses for scleroproteins were done on dehydrated and fat extracted aortas from 10 control and 10 BAPN fed rats. Collagen and elastin were calculated from hydroxyproline concentrations in the soluble and insoluble protein fractions. Test aortas with aneurysmal dilatation and fibrosis frequently weighed up to 100% more than controls from rats of similar age. Appreciable fluctuation in per cent collagen and elastin was observed in control aortas. Increased weight of test aortas was due principally to synthesis of collagen and an unidentified protein. Reductions in elastin per cent in test aortas could be accounted for in most instances to increased synthesis of collagen and unidentified protein. Few test aortas had lower elastin concentrations than observed in any of the controls. This suggests that BAPN feeding may on occasion also depress elastin synthesis. (Supported by a grant from U.S.P.H., A-538.)

3 *Pathology*

PAPAIN INDUCED DYSCHONDROPLASIA IN SUCKLING RATS. Leonard P. Merkow* and Joseph J. Lalich. Univ. of Wisconsin Med. School, Madison, Wis.

Short-term studies following papain injections into animals show a depletion of acid mucopolysaccharides from cartilage. For this reason it seemed desirable to study the effect of repeated papain injections on skeletal development. A 1% crude papain suspension was injected intraperitoneally into 10 day old rats daily for periods of 53-71 days. Four saline controls and thirteen test rats from four litters were injected. This resulted in shortening of the extremities, rigidity of the vertebral column and flaring of the thoracic cage. Widening at its caudal end and an increase in the anteroposterior diameter resulted in a bell-shaped rib cage. Shortening of the extremities was usually associated with a limitation of mobility in animals. Microscopic cartilaginous changes not seen in controls were observed in epiphyseal plates and intervertebral discs of test rats. A significant reduction in the number of epiphyseal cartilage cells was encountered in long bones. Decreased vertebral column mobility was due to destruction of the nucleus pulposus and cartilaginous fusion of vertebral bodies. Repeated papain injections also produced a significant reduction in weight gain. (Supported by grants from U.S.P.H., 2G-130 and A-538.)

4 *Pathology*

LUPUS CELLS AT LOCAL INFLAMMATORY SITES IN LUPUS PATIENTS. Pasquale E. Perillie, Paul Calabresi, and Stuart C. Finch. (Intr. by Harry S.N. Greene) Yale University School of Medicine, New Haven, Connecticut.

In the process of studying the local inflammatory response in patients with systemic lupus erythematosus (SLE), lupus cells were detected. This study was undertaken to determine the pattern of lupus cell production in these patients. A group of five patients with SLE was studied utilizing the skin window technique of Rebuck. This technique consists of the serial application of sterile coverslips to superficial abraded lesions on the volar surface of the forearm. The coverslips were removed at timed intervals and stained with Wright-Giemsa. Lupus cells were produced in two patients, 3-5 hours following the initial local cellular response. Earlier preparations demonstrated some of the cytolytic processes leading to the production of lupus cells. Lupus cells could not be demonstrated beyond the 5 hour stage in these patients. Of the five patients studied only the two yielding positive results were in an active phase of their disease and had positive in vitro lupus preparations. This study demonstrates that lupus cells are produced at sites of leukocyte stagnation in patients with SLE and may be useful in studying the pathogenesis of the lupus phenomenon.

5 *Pathology*

NITROGEN BALANCE IN LATHYRITIC RATS. Cedric McCrary* and J. L. Orbison. Department of Pathology, University of Rochester School of Medicine, Rochester, N. Y.

Three groups of weanling rats, each of equal numbers, were fed ground Purina Chow. To the diet of the experimental animals 0.4% of Beta-aminopropionitrile fumarate (BAPN), a potent lathyrogen, was added. Each animal was housed in an individual metabolism cage. The animals of one control group were pair-fed with those of the experimental group, while the animals of the other control group were allowed to eat ad libitum. Serial collections of feces and urine were made and assayed for total nitrogen content. Food intake was measured daily and the total daily nitrogen intake calculated for each animal. The rats on the BAPN diet retained less nitrogen than either the pair-fed or ad libitum controls, but at all times remained in positive nitrogen balance. This trend was noted as early as 24 hours after including the BAPN in the diet. Throughout the 16 days of the experimental period no significant differences in body weights of BAPN and pair-fed control rats occurred. The data indicate that BAPN alters nitrogen metabolism by inducing a shift in balance toward nitrogen catabolism even when dietary intake is equalized by pair-feeding. (Supported by grant E-1455 (C1) from the U.S.P.H.S.)

089. CONNECTIVE TISSUE—Metabolism

1 *Biochemistry*

STUDIES ON THE SOLUBILIZATION OF NATIVE COLLAGEN. Norman H. Grant and Harvey E. Alburn*. Wyeth Institute for Medical Research, Radnor, Pa.

Collagen is sometimes rapidly metabolized in such tissues as bone, uterus, and healing wounds, but the mechanism has been elusive. We have examined the solubilization of washed, dissected native collagen fibers from rat tail tendons at 38° and pH 7.4 under the influence of chemicals which collagen might encounter in vivo. Calcium salts alone are effective solubilizing agents. Moreover, they appear to render collagen susceptible to proteolysis by trypsin, chymotrypsin, elastase, and the endopeptidase from pro-carboxypeptidase. Arginine and creatinine potentiate the combined action of Ca^{++} and chymotrypsin. Salicylates alone induce solubilization, and, as with Ca^{++}, they prime collagen for proteolysis and are potentiated by arginine. Unlike Cl. histolyticum collagenase activity, the observed mammalian proteolysis of collagen is not inhibited by cysteine. While lysine somewhat lowers Ca^{++}-activated digestion, lysine ethyl ester increases it. Collagen from old rats is more resistant to enzymatic solubilization than that from young rats of the same strain. The adsorptive capacity of collagen makes it likely that adequate concentrations of solubilizing agents may occur locally in vivo.

2 *Biochemistry*

THE HYDROXYLATION OF PROLINE AND ITS INCORPORATION INTO COLLAGEN. E. Hausmann (intr. by W. F. Neuman), Depts. of Radiation Biology and Biochemistry, Univ. of Rochester, School of Medicine and Dentistry.

A comparison was made of the specific activities of collagen incorporated proline and hydroxyproline after administration of C^{14} proline. 18 guinea pigs (280-350 grs) were each injected intracardially with 25 μc of C^{14} proline; two animals were sacrificed at each 3 hour interval during the first 24 hours. Neutral, soluble collagen was extracted from the skins, purified and acid-hydrolyzed. Proline and hydroxyproline were isolated by paper chromatography. The ratio of the specific activity hydroxyproline/proline at every time interval did not significantly differ from unity. Enzymatic hydrolysis of collagen with collagenase (Grant and Alburn, Arch. Biochem. Biophys. 82, 245, 1959) gave similar results. No significant radioactivity was found in proline when C^{14}-proline was added in vitro to unlabelled skin. The conclusions drawn from this study are: 1. The specific activity of proline was not influenced by free adsorbed proline nor by proline from a non-collagenous protein source. 2. The hydroxylation of proline is closely related in time to its incorporation into collagen. (Supported by Grant DT3, National Institute of Dental Research.)

XUM

090. CONNECTIVE TISSUE– Mucopolysaccharides

3 *Biochemistry*

METABOLISM OF CHONDROITIN SULFATE B. Eugene A. Davidson. Department of Biochemistry and Center for the Study of Aging, Duke University, Durham, North Carolina.

Crude extracts of rabbit skin catalyze the incorporation of inorganic sulfate into acceptor substrates prepared by chemical desulfation of chondroitin sulfate A or chondroitin sulfate B. The overall process is ATP dependent, mediated through the formation of phosphoadenosine phosphosulfate and, in partially purified preparations, stimulated 2-3 fold by the presence of 2×10^{-4}M UTP but not GTP or CTP. Chondroitin B is a more efficient acceptor than chondroitin A whereas hyaluronic acid, chondroitin sulfate A or B and keratosulfate are inactive. The mechanism of transfer has not been established. When S-35 labeled chondroitin sulfate B is incubated with crude extracts of rabbit skin, there is a disappearance of mucopolysaccharide and the concomitant formation of products with differing paper electrophoretic mobility. There is no release of inorganic sulfate (less than 0.01%) nor is there any detectable free iduronic acid or acetylgalactosamine. The formation of these apparent degradation products can be prevented or reversed by the presence of 10^{-4}M UTP. Comparable transformations are not observed with chondroitin sulfate A as a substrate.

4 *Biochemistry*

SOME ASPECTS OF COLLAGEN METABOLISM. Georg Gerber,* Gisela Gerber,* L.H. Hempelmann* and K.I. Altman. Univ. of Rochester School of Med., Rochester, N.Y.

Growing rats were injected with proline-U-C^{14}. Proline and hydroxyproline from fractions of skin collagen as well as several urinary excretion products derived from hydroxyproline residues were isolated and the respective specific activities determined. Proline from nonfractionated skin collagen has a turnover time of 65 days. The specific activity of hydroxyproline increased initially and then decreased. Skin collagen was fractionated into insoluble and citrate soluble fractions. The turnover time of proline in the former fraction (>200 days) exceeded that of the latter fraction (16 days). The continuous increase in the specific activity of hydroxyproline isolated from insoluble collagen suggests that formation of insoluble collagen continues for a much longer time than that of citrate soluble collagen. The specific activity - time curve for "free" hydroxyproline in the urine suggests the presence of metabolic components with a short (1day) and a long (6 days) turnover time, respectively. The curve of C^{14}-activity of pyrrole carboxylic acid in the urine also shows the same two components. Urinary "bound" hydroxyproline has only the component having the longer turnover time. This investigation was supported in part by the USAEC, the USPHS and the Max Kade Foundation.

5 *Biochemistry*

THE SITE OF CAPSULE (HYALURONIC ACID) SYNTHESIS IN GROUP A STREPTOCOCCUS. A. Markovitz* and A. Dorfman. LaRabida Institute and Depts. of Microbiology and Pediatrics, U. of Chicago.

Hyaluronic acid (HA) synthesis has been demonstrated with a particulate enzyme obtained from Group A Streptococcus after sonic disruption (J. Biol. Chem., 234, 2343, 1959). Analyses demonstrated cell wall components and suggested the activity resided in adhering membranes. Group A streptococci protoplasts were prepared (J. Exp. Med., 110, 853, 1959) and disrupted in hypotonic media. Centrifugal fractionation revealed: 1) all enzymic activity sedimented at 105,000 x g; 2) 74% sedimented in 30 min. at 30,000 x g (electron microscopy revealed chiefly membranes, less than 9% of the total rhamnose was in this unwashed fraction); 3) another membrane preparation, obtained by centrifugation at 11,000 x g for 30 min. retained enzyme activity after treatment with DNase and RNase and resedimentation under the same conditions. This infranatant fraction contained 55% and the supernatant digest 24% of the total enzymic activity. The sum (79%) of the activities of the supernatant and infranatant fractions approximates (74%) that of a preparation of membranes not treated with DNase and RNase, indicating these enzymes have little effect on HA synthesis. Thus, HA synthesis is associated with the protoplast membrane. Enzymes responsible for capsule synthesis in other bacteria also may be associated with the cell membrane.

STRUCTURE OF KERATOSULFATE. S. Hirano*, P. Hoffman and K. Meyer. Dept. of Medicine, Columbia Univ. College of Phys. and Surg., New York.

Keratosulfate of bovine cornea and its desulfated polymer were methylated, the resulting methyl ethers hydrolysed, and the following crystalline compounds isolated: 2,4-di-O-methyl-D-galactose anilide, 2,4,6-tri-O-methyl-D-galactose, 2,3,4,6-tetra-O-methyl-D-galactose anilide, 2-acetamido-2-deoxy-3-O-methyl-D-glucose, and 2-acetamido-2-deoxy-3,6-di-O-methyl-D-glucose. Comparison of the relative hypoiodite values of the methylated monosaccharides from sulfated and desulfated polysaccharides indicated that the sulfate was attached to the glucosamine moiety. The results of these studies combined with enzymatic hydrolysis indicated that the main structure of keratosulfate contains the repeating unit 4-O-β-D-galactopyranosyl-3-O-(2-acetamido-2-deoxy-β-D-glucopyranosyl-6-O-sulfate). (Supported by grants from the National Science Foundation, the U.S. Public Health Service, and the Edward Daniels Faulkner Arthritis Clinic of the Presbyterian Hospital New York.)

2 *Biochemistry*

SODIUM CHONDROITIN SULFATE-PROTEIN COMPLEXES OF CARTILAGE. III. COMPLEXES FROM SHARK. Martin B. Mathews. LaRabida Institute and Dept. of Biochem., U. of Chicago, Chicago, Ill.

Composition and macromolecular structure of protein complexes containing chondroitin sulfate C have been revealed by techniques previously applied to the chondroitin sulfate A-protein complexes of mammalian cartilage. The "C" complexes contain 14-16% protein which resembles in amino acid composition the non-collagenous protein of the "A" complexes. The mucopolysaccharide moiety has a molecular weight range of 40 to 50 thousand. High molecular weight aggregates of complex are composed of basic units of 4 million molecular weight. Compared to the "A" complexes, the "C" complexes have greater stability towards heat and urea, possibly a reflection of the high urea content of shark tissues. The remarkable similarity of "C" and "A" complexes suggests the existance of a common plan of molecular evolution with minor structural variations arising in response to specific adaptive requirements. The finding of an acid mucopolysaccharide identical with (or closely similar to) keratosulfate in small amounts in shark cartilage indicates the probable evolutionary antiquity of keratosulfate. (Supported by grants from The American Heart Association and The National Foundation).

3 *Biochemistry*

STUDIES ON CHONDROITINASE FROM P. VULGARIS. H.I. Makada, J.B. Wolfe and Silvia Garcia*, Scripps Clinic and Res. Fdn., La Jolla, Calif. and U. South. Calif. School of Medicine, Los Angeles.

Chondroitinase purified from Proteus vulgaris rapidly depolymerized chondroitin sulfate. Recent data indicate that the product of this reaction is similar in structure to the unsaturated disaccharide that is produced by the action of bacterial hyaluronidase on hyaluronate rather than N-Acetylchondrosin sulfate. A new assay system based on the u.v. absorption of the product at 232 mμ has been devised. Using this method, the effect of various salts at differing ionic strength has been studied. In all cases, as the ionic strength is increased, the reaction first reaches a maximum, then becomes inhibitory. The effect of anions in order of decreasing activity were: Acetate, F^-, Cl^-, Br^-, AsO_4^-, glucuronate, malonate, citrate, $SO_4^{=}$, $HPO_4^{=}$, formate, AsO_2^-, HCO_3^-, CN^-, $B_4O_7^-$. The effect of cations in decreasing activity were: Mn^{++}, Mg^{++}, Tris$^+$, Na^+, K^+, Ba^{++}, NH_4^+, Co^{++}. Sucrose or glucose had no effect at low concentrations and was inhibitory at the higher ranges. The effects were not additive but inhibitory with the ions showing the least activation effect being the better inhibitors. The pH maximum using various salts remained fairly constant at pH 7.9 to 8.0. The ion activations appear to differ from those reported for amylase and arylsulfatase. (USPHS grant A-1645.)

4 *Biochemistry*

ENZYMATIC PRODUCTION OF A KETO ACID FROM HYALURONATE. Alfred Linker and Phyllis Sampson*. Columbia University College of Physicians and Surgeons, New York, New York.

Extracts of flavobacterium degrade hyaluronate and chondroitin sulfates A and C. With hyaluronate an "unsaturated disaccharide," identical with that produced by pneumococcal hyaluronidase, is obtained. Extracts of organisms adapted to chondroitin sulfate A also degrade hyaluronate. When these extracts were used, the carbazole reaction for uronic acids decreased with incubation time. Paper chromatograms of hyaluronate digests showed the presence of N-acetylglucosamine and an unidentified compound. Identical products were obtained when "unsaturated disaccharide" was incubated with extracts of the adapted organisms, while N-acetylhyalobiuronic acid remained unchanged. The unidentified compound was isolated as the Ca salt. It gave semicarbazide and diphenylamine reactions typical for α-keto acids. It was decarboxylated by ceric sulfate and precipitated with 2,4-dinitrophenylhydrazine. Therefore, the extracts of adapted flavobacteria contain an enzyme which splits the uronidic bond of Δ-4,5 unsaturated disaccharides, yielding N-acetylglucosamine and an α-keto acid, explaining the apparent loss of uronic acid. (Supported by grants from the American Heart Association and the U. S. Public Health Service.)

5 *Biochemistry*

ENZYMATIC HYDROLYSIS OF KERATOSULFATE. O. Rosen*, P. Hoffman, and K. Meyer. From the Dept. of Medicine, Columbia Univ. College of Physicians and Surgeons, New York.

Keratosulfate of cornea and rib cartilage is composed of N-acetylglucosamine, D-galactose, sulfate, and 1 to 2% of a methylpentose. Composition and cross reactions with antiblood group sera indicated a similarity of keratosulfate to blood group substances. Among several enzymes which degrade blood group substances, those contained in extracts of a coccobacillus (Chase) hydrolysed keratosulfate to oligosaccharides, sulfate, D-galactose, and N-acetylglucosamine. Formation of the monosaccharides was due to the presence of exo-β-galactosidase and exo-β-glucosaminidase, establishing the β nature of both glycosidic linkages. The enzymes could be partially separated by $(NH_4)_2SO_4$ fractionation. (Supported by grants from the National Science Foundation, the U. S. Public Health Service, and the Edward Daniels Faulkner Arthritis Clinic of the Presbyterian Hospital, New York.

6 *Biochemistry*

STUDIES ON SIALIC ACIDS. L. Warren and S. S. Spicer (intr. by K. Kurahashi). Natl. Insts. of Health, Bethesda, Md.

Sialic acids have been further studied in various tissues using the thiobarbituric acid assay (L. Warren, J. Biol. Chem. 234, 1971, 1959). (1) In agreement with previous studies most sialic acids are bound in large molecules. However we have found significant quantities of free sialic acids in trout eggs (35 out of a total of 70 μg./egg) and in human urine (3-5 mg. %). Both N-glycolyl and N-acetylneuraminic acids are found in one cell such as the trout egg. (2) The removal of sialic acids from polymers has been investigated using purified preparations of sialidase from Clostridium perfringens and V. cholera. Marked differences in susceptibility of mucins to sialidase have been found. For example sialidase removes sialic acids from mouse sublingual gland mucins but not from rat sublingual mucin and other rat mucins. (3) A histochemical technique has been developed using basic dyes such as Alcian blue or Azure A to localize sialoproteins. This method has been applied to the identification of sialomucins in mouse salivary and laryngeal glands, vaginal epithelium, and rat thyroid cancers. Basophilic staining is prevented by preliminary methylation or by treatment of sections with sialidase.

7 *Biochemistry*

FRACTIONATION OF HYALURONIC ACID FROM THE BOVINE VITREOUS BODY T. Laurent, A. Pietrusskiewicz and M. Ryan (intr. by L. Varga) Retina Foundation, Mass. Eye and Ear Infirmary, Boston.

A method has been developed for a molecular weight fractionation of bovine vitreous hyaluronic acid (HA). HA was isolated from vitreous bodies by cetylpyridinium chloride(CPC) precipitation at low ionic strength. Dissolving the precipitate in 2M NaCl and treatment with Micro-Cel E effectively removed CPC. The HA had a mol.wt. of 4×10^6 as measured by light-scattering, while sedimentation and diffusion studies gave a value of 2.5×10^6. The stepwise dilution of a solution of HA and CPC in .25M Na_2SO_4 to lower salt concentrations yielded precipitates, which when dissolved and freed from CPC constituted the fractions studied. The individual fractions had mol.wts. ranging from 8×10^4 to 1.7×10^6. The good agreement between the mol.wts. obtained by light-scattering and ultracentrifuge methods for these fractions indicated that the discrepancy noted in the unfractionated material was due to polydispersity. A linear relationship between log limiting viscosity number (ml/g) and log weight-average mol.wt. obtained by light-scattering was found ($[\eta] = .036M^{0.78}$). The results suggest the presence of two species of HA in the bovine vitreous body, one with an average mol.wt. of about 1.5×10^6 and one with an average mol. wt. below 4×10^5. (Supported by a grant(B-1146) from the National Institute of Neurological Diseases and Blindness, PHS.)

8 *Biochemistry*

BOVINE SUBMAXILLARY AND SUBLINGUAL MUCINS. S. Tsuiki*, Y. Hashimoto* and W. Pigman. Univ. of Alabama, Med. Center, Birmingham.

Mucins have been isolated by mild procedures from aqueous extracts of bovine submaxillary and sublingual glands. The sublingual mucin was obtained by successive precipitations at 2-5° with ammonium sulfate (at 10 to 20% saturation), sodium sulfate (2.3 to 6.5%) and calcium chloride in 60% ethanol. The submaxillary mucin was obtained by electrodeposition and subsequent ammonium sulfate fractionation. Physical methods indicated that no appreciable degradation occurred during the preparations. The products dissolved to give clear, viscous solutions. Bovine submaxillary mucin contained about one-third peptides, and the remainder was composed of equimolar ratios of sialic acid and galactosamine. Bovine sublingual mucin generally had a composition much like known blood group substances. The best product, probably still not pure, contained less than 20% of protein. The carbohydrate portion was comprised of the following molar ratios of sugar units: D-galactose (2), hexosamine(galactosamine and glucosamine) (2), sialic acid (1) and L-fucose (1). By digestion with trypsin at 37°, both mucins rapidly lost their viscous nature, but no hexosamine or sialic acid appeared in dialysates. (Supported by National Institutes of Health and Army Surgeon General.)

9 *Biochemistry*

PREPARATION OF TWO DIFFERENT GLYCOPEPTIDE FRACTIONS FROM HUMAN GAMMA GLOBULIN. Z. Dische and O. Gabriel* Coll. of Phys. & Surg., Columbia Univ., New York.

Glycopeptides have been prepared from fractions $II_{1,2}$, II_3, IV, IV_1, III, and III_0 of Cohn from human plasma and from rat kidney proteins by extraction with 80% alkaline ethanol at R.T. Total mucopolysaccharide in these preparations was also fractionated by extraction with 10% TCA at 90° C. during varying time intervals. The analysis of fractions from all preparations showed in those extracted by TCA in the first 6 hours a mannose to galactose ratio similar to those in glycopeptides extracted with alkaline ethanol. In all fractions extracted after 6 hours this ratio was 2-3 times as high as in 6 hour fractions, although the hexosamine to hexose ratio remained the same. A glycopeptide containing 60% carbohydrate was prepared from gamma globulin by extracting it first for 6 hours with 10% TCA at 90° C., washing the residue with TCA and ethanol, and extracting it for 48 hours at R.T. with 80% ethanol containing 5% KaOH. The mannose to galactose ratio of this fraction was 5:1 against 2:1 found in fractions extracted by TCA in the first 6 hours or by alkaline ethanol. These findings indicate the presence of two glycopeptides or one highly asymmetric branched one in all these proteins. The asymmetry would reside with the distribution of hexoses and sialic acid along the chains.

XUM

10 *Biochemistry*

METHOD FOR SEPARATION OF MUCOPOLYSACCHARIDES FROM RAT SKIN. Sara Schiller and Gwendolyn Slover*. LaRabida Institute and Dept. of Biochem., U. of Chicago.

Procedures have been devised for the extraction and separation of hyaluronic acid, chondroitinsulfuric acid and heparin on a quantitative basis from rat skin. These methods have made possible routine detection of small amounts of heparin, its isolation with ease and certain metabolic studies, including the effect of ageing. Crude mucopolysaccharides were prepared by a modification of a method published previously (J. Biol. Chem., 211, 717, 1954). The mucopolysaccharides are then completely precipitated with cetyl pyridinium chloride (CPC) in the presence of 0.04 M NaCl. Celite is added to absorb the mucopolysaccharide-CPC complex. After centrifugation, the supernatant is decanted into the first of a series of tubes or flasks, depending upon the size of the sample. The Celite is washed once with 0.04 M NaCl in 0.1% CPC by stirring with a glass rod. Hyaluronic acid is removed by several washes with 0.3 M NaCl in 0.1% CPC, each wash being individually maintained. The chondroitinsulfuric acids are similarly extracted with 1.2 M NaCl in 0.1% CPC. Further extractions with 2.1 M NaCl remove heparin. 80 to 100% of mucopolysaccharides added to rat skin can be recovered. The method offers a simple and sufficiently quantitative means of screening small quantities of normal and pathological tissues.

11 *Biochemistry*

STUDIES ON MOLECULAR SIZE OF HYALURONIC ACID USING PERIODATE OXIDATION. James C. Melby*, Marlys St. Cyr*, Abraham Unrau* and Fred Smith* (intr. by James S. Dinning). Depts. Med. and Biochem., Univ. Ark. Med. Ctr., Little Rock, Ark., and Dept. Agric. Biochem., Univ. of Minn., St. Paul, Minn.

A method of periodate oxidation of end groups of linear polysaccharides was applied to hyaluronic acid derived from human and bovine synovial fluid, human umbilical cord and canine vitreous. A hyaluronic acid-protein complex was precipitated from synovial fluid with glacial acetic acid. The precipitate and tissues were treated with hot alkali until the product was protein free (micro-biuret negative). The product was reduced with KBH_4 and oxidized with $NaIO_4$ for 24 and 48 hrs. The solution was dialyzed and formaldehyde generated was measured by the chromotropic acid method. Two moles of CH_2O were generated per end group oxidized. Using an erythritol standard and corn starch control, the degree of polymerization and mol. wt. were calculated. Mol. wt. of hyaluronate from human and bovine sources ranged from 20-50,000. Canine vitreous averaged 7,000. These values are appreciably lower than those obtained by physicochemical methods in which protein is incompletely removed. Degradation of hyaluronate in the isolation procedure has not been excluded as a possibility but preliminary observations do not support this explanation.

12 *Pathology*

URINARY ACID MUCOPOLYSACCHARIDES IN HEREDITARY DEFORMING CHONDRODYSPLASIA (DIAPHYSIAL ACLASIS). Andrew E. Lorincz (intr. by R. T. Smith.) Univ.of Florida School of Medicine, Gainesville, Florida.

The urinary excretion of acid mucopolysaccharides was studied in two families with this dominantly transmitted abnormality of bone and cartilage development. Gross quantitative estimations of acid mucopolysaccharide excreted were performed using the acidified bovine serum albumin turbidity technique previously used in studying the acid mucopolysaccharide excretion in the Hurler syndrome. Affected individuals in both families excreted quantities of acid mucopolysaccharide comparable to that excreted by individuals with the Hurler syndrome. The turbidity producing material in the urine of patients with heritable deforming chondrodysplasia tends to dialyze slowly through cellophane casing.

Preliminary study of the properties and chemical analyses of appropriately isolated polysaccharide from these urine samples indicate that it is similar to chondroitin sulfuric acid A or C. Studies to more accurately identify the mucopolysaccharide(s) excreted in the urine as well as those present in a surgically excised exostosis are in progress. These findings would indicate that this condition represents another heritable disorder of connective tissue acid mucopolysaccharide metabolism such as the Hurler syndrome and Marfan's syndrome.

13 *Pathology*

THE DISTRIBUTION OF MAMMALIAN TISSUES OF AN ENZYME SYNTHESIZING GLUCOSAMINE 6-PHOSPHATE. Robert E. Priest. Dept. of Pathology, Univ. of Washington, Seattle, Wash.

An enzyme synthesizing glucosamine-6-phosphate from hexose 6-phosphate and glutamine has been described both in fungi and certain mammalian tissues, and is considered to be important in the synthesis of mucopolysaccharides. The failure to find stimulation of incorporation of S^{35}-sulfate into cartilage or aortic tissue of rats when incubated in the presence of glutamine prompted an investigation of the distribution of this enzyme in several tissues in which mucopolysaccharide synthesis would be expected. Tissues from young adult male and female rats, rabbits, guinea pigs, and humans were used. Synthesis of glucosamine was readily demonstrable in homogenates of liver, kidney, lung and mucous-secreting epithelium. No activity was found in arterial tissue, cartilage from several sites or nucleus pulposis. Colonic mucosa was about ten fold more active per gram of tissue nitrogen than was liver. No significant differences between species or between sexes was found. The failure to demonstrate this enzyme in tissues such as cartilage, aorta and pulmonary artery, where mucopolysaccharide synthesis occurs at a high rate, suggests that it may not be the source of hexosamine for the synthesis of all mucopolysaccharides. (Supported by a grant from the Life Insurance Medical Research Fund)

14 *Pathology*

REACTIVATION IN VIVO AND IN VITRO OF IODOACETAMIDE-TREATED PAPAIN. G. Weissmann*, J.L. Potter*, R.T. McCluskey and L. Thomas. New York Univ. College of Med., New York.

In rabbits, widespread depletion of cartilage matrix occurs following an I.V. injection of crystalline papain in excess iodoacetamide. This mixture is devoid of proteolytic activity in vitro, even after addition of cysteine, and the protease is generally regarded as being permanently inactive. However, it has been shown that following removal of excess iodoacetamide by dialysis, papain retains some activity demonstrable in vitro by (1) Hydrolysis of $HSAI^{131}$ in the presence of cysteine and versene, (2) Release of chondroitin sulfate from isolated cartilage, (3) Reduction of viscosity of solutions of polysaccharide complex from bovine nasal cartilage, (4) Breakdown of C.M.P., determined by differential alcohol precipitation, chromatography, and centrifugal analysis. This residual in vitro activity is attributable to a small fraction of the papain which apparently survives exposure to iodoacetamide in a state of reversible oxidation instead of undergoing alkylation and permanent inactivation. It is suggested that following I.V. injection of a mixture of papain and iodoacetamide, redistribution of the components in effect removes the excess of iodoacetamide in vivo, permitting reactivation of the small, residual fraction of papain which then initiates the changes in cartilage matrix.

091. CONNECTIVE TISSUE—Wound Healing

Biochemistry

EFFECT OF WOUNDING ON UPTAKE OF SULFUR AMINO ACIDS BY LEUCOCYTES. Martin B. Williamson, M. V. Whalen* and H. B. Haley*. Loyola Univ., Schl. of Med., Chicago.

Proteins in regenerating wound tissue accumulate much more cystine than methionine. Histological examination of this tissue reveals the presence of very many leucocytes. To discover whether these facts are related, 30 ml of blood (containing PVP and heparin) were incubated with 3.9 μcuries of radiomethionine or radiocystine. After the erythrocytes had settled, the white cells were centrifuged out of the residual plasma and washed several times with 0.16 N NaCl and 0.1 M acetic acid. The cells were then hydrolyzed in 0.1 N NaOH at 95° C. Radioactivity and tyrosine were determined in the hydrolysates. The tyrosine level was used as the measure of white cell content. The leucocytes of normal dogs and those from dogs wounded 3 or 7 days previously incorporated the same amount of methionine-S^{35}. However, cystine-S^{35} was taken up more rapidly by leucocytes from wounded than from unwounded dogs. About 5 times as much radiomethionine as radiocystine was incorporated by the white cells. (This work was supported by a grant from the U. S. P. H. S.).

092. ENDOCRINES

2 *Biochemistry*

SYSTEMIC RESPONSE OF COLLAGEN TO LOCAL INFLAMMATION. John C. Houck and Raymond A. Jacob.* Biochemical Lab. Children's Hospital Research Foundation, Washington, D.C.

The effect of various concentrations of dermally injected croton oil upon the local and systemic loss of collagen associated with inflammation was studied in male rats by the following chemical changes in dermal hydroxyproline, hexosamine and nitrogen. At the site of injury the skin hydroxyproline content decreased 75% with 50% croton oil induced necrosis 60% with 75% oil and 45% with 100% oil. Eschars formed 3, 5, and 7 days and sloughed 11, 14 and 17 days after the respective injuries. The total hydroxyproline of the uninjured skin showed 25% decrease for all concentrations of irritant. Only with the 100% croton oil injury was found grossly increased vascularization and sera containing hydroxyproline polypeptides. The concentration of insoluble collagen in the uninjured skin sequentially fractionated with .15 M NaCl, .5 M NaCl and citrate buffer (pH 3.6) closely paralleled the total systemic decrease in collagen. A transient increase in both saline and citrate soluble collagens was found to be inversely related to the total systemic collagen change of the uninjured skin. These results seem to indicate that systemic insoluble collagen is transformed into soluble collagen which drains into the lymphatic where it is retained.

3 *Biochemistry*

WOUND HEALING: GLYCOPROTEINS OF EXPERIMENTAL WOUND TISSUE AND FLUID. M. R. Shetlar, Danny L. Hern, Betty N. White and John A. Schilling (Introduced by Fay Sheppard.) Univ. Oklahoma School of Med., Oklahoma City, Okla.

Serial studies of the glycoproteins and mucopolysaccharides were made during the wound healing process. The technic of Schilling et. al.(Proc. Soc. Exp. Biol. and Med. 89, 189, 1955) utilizing the implantation of stainless steel wire mesh cylinders was used. By this technic, composition of the connective tissue formed within the cylinder may be compared with that of the fluid in contact with the tissue. As indicated by paper electrophoretic studies of fractions from saline and alkaline extracts, two acid mucopolysaccharides could be distinguished in the tissue by the second week after implantation. One of these exhibited metachromasia with toluidine blue and had the proper mobility for chondroitin sulfate. In the fluid only small amounts of acid mucopolysaccharides were found in one and two week samples. However, at 3 weeks, two strong electrophorestic bands were noted, one of which had the mobility and staining characteristics of chondroitin sulfate. Changes of the composition of the wound tissue with time were also indicated by quantitative determinations of hexose, hexosamine and uronic acid.

4 *Biochemistry*

A Role for Ascorbic Acid in the Maintenance of Newly-Formed Collagen. Bernard S. Gould, Div. Biochem., Dept. Biol., M.I.T., Cambridge, Mass.

Although there is little question that a relationship exists between ascorbic acid and collagen formation it is not clear that ascorbic acid is involved in the maintenance of collagen. This problem has been studied in young guinea pigs by the polyvinyl sponge implantation method. Collagen has been allowed to form for varying periods in subcutaneously implanted sponges in ascorbic acid depleted and undepleted animals. After this time the animals were placed on a scorbutigenic diet. Determinations have been made of the amounts of 0.45 M NaCl soluble collagen and of insoluble collagen at varying periods after implantation and withdrawal of ascorbic acid. Highest yields of soluble collagen accompany active synthesis. Definite and very prompt disappearance of both soluble and insoluble collagen, as determined by hydroxyproline analyses occurs particularly in animals depleted before implantation. As much as 75% collagen disappears in 30 days. Young collagen is most rapidly removed. The hydroxyproline:proline:glycine ratios of the purified 'collagen' remaining after extensive resorption, when compared with the ratios found in normal collagen, suggest that collagenolysis is involved.

1 *Physiology*

MAMMARY GLAND STATUS IN DECLINING LACTATION. Richard C. Moon (Intr. by R. H. Alden) Department of Physiology, University of Tennessee, Memphis.

Milk yield in the rat increases markedly after parturition, reaches a maximum at approximately 14 days and then gradually declines. In order to ascertain whether decline in milk yield is associated with changes in cell number, total desoxyribonucleic acid (DNA) of the abdominal-inguinal glands of primiparous lactating rats was employed as an estimate of the number of cells present during early (day 1), intense (day 14), and declining (day 28) lactation. Rats of the declining lactation group were provided with 4 day old foster litters on day 14 of lactation. Milk yield of each rat was determined immediately prior to sacrifice. Total DNA of glands of rats sacrificed on day 14 was greater than that of glands from animals killed on day 1 or 28 of lactation. A highly significant correlation existed between total DNA and milk yield. These data suggest that mammary growth continues well into lactation and that decreased milk yield may be due, in part, to a reduction in the number of mammary gland cells.

2 *Physiology*

EFFECT OF PARATHYROID HORMONE (PTH) ON CITRATE PRODUCTION. J.C. Schooley and E. Otero.(intr. by H.B. Jones) Donner Lab., Univ. of California, Berkeley.

The role of citrate (Ci) in the mediation of PTH induced bone resorption has been studied using the isolated perfused hind-limb of the rat. 1 hr. after the addition of 100 units of PTH the total plasma Ci was 2-3 times that found in control perfusions whereas lactate increased only slightly. Total plasma phosphate did not change, but an increase in calcium was seen in some experiments. The Ci increase after PTH would have been sufficient to produce about an 8-10% decrease in bone Ci if PTH simply caused a release of Ci from bone; however, no differences were found in the Ci content of the bone of the perfused limb when compared to the non-perfused limb of the same rat. We conclude, therefore, that the increased Ci is due to the metabolism of the tissues of the perfused limb. The finding(Neuman et al., J.Am.Chem.Soc.78:3863, 1956)that after PTH administration the Ci content of femoral blood was less than blood directly draining bone, suggests that much of the increased Ci production after PTH was the result of bone cell activity; but some formation by muscle,skin,and marrow cannot be excluded.

3 *Physiology*

EFFECT OF GLUCAGON ON AMINO ACID METABOLISM, R. M. Bocek*, R. D. Peterson and C. H. Beatty. Dept. of Biochemistry, Univ. of Oregon Medical School, Portland, Oregon.

Depancreatized rats had higher plasma amino acid levels than control, alloxan diabetic or sham operated animals. Plasma amino acids of pancreatectomized rats were decreased 2 hours after the second dose of crystalline glucagon (0.1 mg per 200 gm rat at 4:30 p.m. and 8:30 a.m.). In further studies, rat adductor muscle fibers in vitro were used (Krebs phosphate buffered medium). Amino acid release (the difference between the amino acid nitrogen in the medium after 15 minutes and 135 minutes) by fibers from control or depancreatized rats was not changed by the addition of insulin (0.5 units/ml medium). Glucagon (20 ug/ml) in addition to insulin did not change the amino-N release of fibers from control rats (+1.5±1.2 SE ug/100 mg/hr, n = 10) but did increase release in the diabetic series (+3.0±1.0 ug/100 mg/hr, n =13). Another series of experiments was done in HCO_3^- buffer. The addition of 0.12 ug/ml of insulin had no effect on amino-N release from fibers of control rats (- 0.04 ± 0.2 ug/100 mg/hr, n = 9) but 20 ug of glucagon/ml increased amino N release (+1.7± 0.5 ug/100 mg/hr, n = 10). These data are evidence in support of a catabolic action of glucagon on muscle protein. Aided by Muscular Dystrophy Assn. and PHS grants 1157 and 213.

XUM

4 *Physiology*

IN VITRO PROGESTERONE SYNTHESIS BY SWINE CORPORA LUTEA. G.W. DUNCAN*, W.R. HEARN* AND R.M. MELAMPY. Ia. State Univ., Ames, Ia.

Corpora lutea slices suspended in Krebs-Ringer bicarbonate buffer medium containing glucose are incubated for 2 hours at 37.5°C under 95% O_2, 5% CO_2. Following incubation progesterone is isolated and quantitatively estimated by a method based upon 95% ethanol extraction, column adsorption chromatography, countercurrent distribution, paper chromatography and ultraviolet absorption spectrophotometry. Initial endogenous and post-incubation progesterone concentrations (mcg/gm) of corpora from gilts 4,8,12,16, and 18 days post estrus are respectively: 20.5, 158.0; 40.4, 113.2; 61.3, 134.5; 74.2, 146.0; 0, 0. For animals pregnant 16, 24, 48, 72, 96, and 102 days the concentrations are: 81.7, 114.2; 83.9, 144.8; 104.5, 140.9; 68.01, 105.5; 52.0, 97.7; 21.1, 68.3. Addition of DPN and pregnenolone to the medium increases in vitro synthesis whereas addition of HCG, PMS, LTH, oxytocin, relaxin or homogenized gilt pituitary tissue has no effect under these experimental conditions. Intramuscular administration of LTH (125 and 250 I.U./day) initiated 12 days post estrus did not alter the time of occurrence of the succeeding estrus. Incubation media was prepared by buffering the fluid expressed from gilt endometrium. Media prepared from uteri obtained at day 18 of the cycle inhibit synthesis whereas media prepared from uteri obtained during pregnancy and early diestrum stimulate synthesis.

5 *Physiology*

THE SPLEEN IN RELATION TO HORMONAL ACTIVITY. F.R. Guastella, L.J. Treciokas, and S.F. Marotta (intr. by S.R.M. Reynolds). Aeromedical Laboratory and the Dept. of Anatomy, College of Med., Univ. of Ill., Chicago, Ill.

Male chicks were splenectomized under ether anesthesia at 5 days of age. Prior to necropsy at 14 days of age both splenectomized and sham-operated chicks were injected intramuscularly with various hormones for 5 days. The testes of splenectomized non-treated chicks showed hyperplasia of interstitial cells and tubular hypertrophy. FSH given alone or together with LH produced significantly larger testes, as well as greater tubular hypertrophy in the splenectomized chicks than in the sham-operated controls. The effects of PMS, LH, androsterone, progesterone or testosterone on the testes and comb were similar in both groups of animals with the exception of androsterone which was more effective in accelerating comb growth in the control chicks. Splenectomy appears to have only a slight effect on adrenal activity. It is postulated that the spleen influences the activities of certain hormones.

6 *Nutrition*

PARATHORMONE-LIKE ACTIVITY IN THE SERA OF RATS WITH INDUCED HYPERPARATHYROIDISM. B. Buckner and J. E. Nellor (intr. by J. A. Hoefer). Michigan State University, East Lansing, Mich.

A hyperparathyroid condition was induced in rats by feeding a calcium-deficient, Vitamin D-free diet. Serum calcium levels were maintained in the 8-11mg.% range during the twenty day trial. Parathormone-like activity was assayed by the rise in serum calcium of thyroparathyroidectomized rats following the injection of sera from the hyperparathyroid rats. Parathormone-like activity was not assayable in the sera of rats until the eighth day of the dietary regime, although serum Ca levels and histological sections of the parathyroid glands and bone indicated that increased activity of the parathyroid glands had occurred by the fourth day of the dietary regime.

A comparison of the serum calcium rise in the assay animals induced by the injection of standard "Parathormone", to that induced by the injection of serum, allowed an estimation of 20 to 43 units of "Parathormone Equivalence" per cc. of serum from calcium deficient rats. A graded parathormone-like response was obtained by varying the amount of serum assayed. Parathormone-like activity was not assayable in 1 cc. of sera from normal rats.

7 *Physiology*

EFFECT OF CORTISONE ON THE MATURATION OF THE GONADS AND SEX RATIO OF RATS. M. White* and E. Da Costa. Chicago Teachers College, Chicago, Ill.

Cortisone was injected twice weekly for 6 to 25 weeks in 6 dose levels ranging from 0.625 to 6.25 mgm. 288 rats were studied; 192 first, 81 second and 15 third generation. Sexual maturity in the 27 control females averaged 52.5 days. All levels of cortisone, except 6.25 mgm which slowed sexual maturity to 56 days, speeded up sexual maturity. The 6 litters from the 0.625 mgm and the 3 from the 6.25 mgm groups were delivered before their control litters. More than half of the litters delivered by rats on the intervening dose levels were delivered before their controls. The sex ratio of 76 males to 74 females of the control litters was reversed for the 1.25 through 3.75 mgm levels with 19 males and 27 females at the last level. At 5 mgm the ratio was 31:15. All experimental females showed a depression in weight. The males increased in body weight from 0.625 to 2.5 mgm and decreased to almost 50% of the control on the 6.25 mgm dose. Body weight decreased further after the first generation. The adrenals showed a definite weight increase at the 2 lowest dose levels and a decrease after that. Thus in general the cortisone stimulated gonadal and somatic functioning at the low dosage levels and depressed functioning at the higher dosage levels, perhaps by way of the pituitary.

8 *Biochemistry*

Quantitative In Vivo Assay of Pineal Melanophore Contracting Principle (Melatonin). E. A. Lerner-Wasem* and Claire E. Graham. The Wilson Laboratories, Chicago.

Administration of pineal extracts produces lightening of skin color, and color change in frogs has been suggested as an assay for pineal extracts (Borg and Ralston, Proc. Soc. Exp. Biol. Med. 77, 807, 1951). Recently, Lerner et al (J. Am. Chem. Soc., 80, 2587, 1958) reported the isolation from pineal glands of the factor (Melatonin) that lightens skin color. The present report describes a quantitative, in vivo assay for melatonin. Frogs (Rana pipiens) kept in jars with a small amount of water were dark-adapted on a dark background under bright overhead illumination. The melanophore index (mi) in the web of the foot was read with 100X magnification. Animals showing fully expanded melanophores, mi of 5.0, were injected with an extract from beef pineals. Mi's were re-determined after treatment. Mean mi's showed a straight line response within an 8-fold dose range at 15, 30 or 60 minutes after treatment. The most rectilinear response, evident over a 16-fold dose range, was obtained when the response was expressed as mi at 15 + 30 + 60/3. Using this expression of response, a 4-point assay using dose groups of 10 frogs showed an index of precision (s/b) of 0.3.

9 *Biochemistry*

A RAPID ACID PHOSPHATASE TEST FOR EVALUATION OF ANDROGEN ACTIVITY. J. E. Kirk. Div. of Gerontology, Washington Univ. School of Medicine, St. Louis, Mo.

In subjects with intact prostatic tissue the acid phosphatase concentration of the prostatic secretion has been found to be a reliable measure of androgen activity. A rapid test for assay of the enzyme activity was developed with the use of phenolphthalein diphosphoric acid as substrate. The sample of secretion obtained by prostatic massage was diluted 1:1000 with 0.06 M potassium acid phthalate buffer, pH 5.0. The substrate solution was prepared by dissolving 0.72 mg. of the substrate contained in a vial in 1.0 ml. phthalate buffer. 0.5 ml. of the diluted prostatic secretion was added to the substrate-buffer solution and allowed to react for exactly 2 minutes at room temperature. At the end of this period, 8.5 ml. of 0.2 M carbonate-bicarbonate buffer solution, pH 10.4 were added, and the quantity of phenolphthalein liberated was determined spectrophotometrically at 550 millimicrons. The reaction was found to be of zero order. The observed K_m for phenolphthalein diphosphoric acid was 3.5×10^{-4} M, and the Q_{10} for the reaction between 20 and 30 C. was 2.25. A close correlation was found between the amount of enzyme present in the sample and the quantity of phenolphthalein liberated.

Biochemistry

10

THYROID-HYPOPHYSEAL RELATIONSHIPS. J.C. Opsahl, D.W. Crocker* and G.J. Dammin* (Peter Bent Brigham Hospital and Harvard Medical School, Boston, Massachusetts)

Previous work by the authors has shown that the regressive changes which occur in the adrenal cortex following hypophysectomy are reversible in that responses can be elicted to placental and pituitary ACTH. The current experiments were designed to determine the effects of hypophysectomy on the thyroid, gonads and viscera as well. Experimental and control rats weighing 240-270 grams, were given ground laboratory chow (Purina) and 5% glucose in water ad lib, but no substitution therapy. Alterations in body weight following hypophysectomy were not significant and during the observation period (6-8 months) the weight remained near the post-operative level. Whereas the adrenal weight fell to 1/3, and the ovarian weight to 1/5 that of the non-operated controls, there was no reduction in the weight of the thyroid. Microscopically, the ovaries and adrenals did not appear irreversibly altered, and the thyroids of the controls and the hypophysectomized rats were similar. It is apparent that the thyroid has been sustained following hypophysectomy either autonomously or by factors not now recognized. Absence of thyroid involution in patients following hypophysectomy for advanced cancer has been noted by Pearson and Gurling. (This study is supported by the Office of Naval Research.)

Biochemistry

11

PARATHYROID HORMONE ACTIVITY OF HUMAN AND RAT PLASMA CONSTITUENTS. M. V. L'Heureux and Leo E. Reichert* Dept. of Biochemistry, Stritch Schl. of Med. and Graduate School, Loyola University, Chicago.

Components of rat and human plasma obtained by ammonium sulfate and cold ethanol fractionation procedures have been assayed for their calcium-mobilizing activity in parathyroidectomized rats. The determinations were made on fractions of rat plasma to which parathyroid extract was added either in vitro or in vivo. Similar experiments were carried out on fractions of human plasma with and without added parathyroid extract. Evidence is presented to suggest that endogenous rat plasma parathyroid hormone activity is normally associated with the α-globulin fraction. Cold ethanol fractionation of normal human plasma and of human plasma to which had been added parathyroid extract, resulted in the localization of the parathyroid activity in the β- and γ-globulin fraction. The normal level of circulating parathyroid hormone activity in human plasma has been estimated to be of the order of 40 U.S.P. units per 100 mls.

Biochemistry

12

7α-HYDROXYLATION OF ADRENAL STEROIDS BY SURVIVING HAMSTER LIVER SLICES. John J. Schneider. Jefferson Medical College, Philadelphia.

Incubation of 11-desoxycorticosterone (I) with surviving hamster liver slices (2 hrs., gas phase oxygen, steroid:tissue ratio 1:1000) followed by paper chromatography of the neutral extract showed that the major UV-absorbing metabolite was a relatively polar steroid which reduced blue tetrazolium and gave a negative soda fluorescence test. This metabolite was isolated in pure form in 25% yield, and identified as 7α-hydroxy-11-desoxycorticosterone by its dehydration in alkali to the readily recognized Δ 4,6-3-one, and by direct comparison with an authentic sample. A degree of substrate specificity was shown in that, under the same conditions, corticosterone gave much smaller amounts of the 7α-hydroxy metabolite, 17-hydroxy-11-desoxycorticosterone only traces and 17-hydroxycorticosterone none. Comparative studies, in which (I) served as the substrate and liver slices from various animals the enzyme source, showed that the ability of guinea pig liver to 7-hydroxylate this steroid is approximately one-fourth that of the hamster and that slices prepared from the livers of the rat, cat, rabbit and pidgeon lack this ability.

Pharmacology

13

THE EFFECTS OF 3,5,3'-TRIIODOTHYRONINE AND SODIUM SALICYLATE ON METABOLISM, PULSE RATE AND BODY TEMPERATURE. C.L. Gemmill, R.G. King* and K.M. Browning*, University of Virginia, Charlottesville, Va.

In two series of experiments, the first using 4.1 micrograms T-3 and 15.7 mgm. sodium salicylate and in the second, 8.9 micrograms T-3 and 26.4 mgm. sodium salicylate per 100 grams of body weight, there was very little change in metabolism, pulse rate or rectal temperature with sodium salicylate alone but there was the usual rise in these three variables on T-3. When the two compounds were injected at the same time, there was a rise in basal metabolism comparable to the T-3 produced rise but there was no increment in pulse rate and body temperature. In a third series, the injection of 12.2 mgm. sodium salicylate per 100 grams in rats made previously hypermetabolic by 32 micrograms of T-3 in 100 ml. drinking water there was no change in the hypermetabolism but there was a fall of pulse rate and rectal temperature to normal limits. These results demonstrate that sodium salicylate abolishes the rise in pulse rate and rectal temperature produced by T-3 but does not affect metabolism.

Pharmacology

14

ENDOCRINE FACTORS IN HISTAMINE SUSCEPTIBILITY. Arthur M. Sackler, Joseph Gennis, P. Jurtshuk, Jr., A.S. Weltman and M. Bradshaw (intr. by R.H. Cheney.) Lab. for Therapeutic Research, Long Island Univ., Brooklyn College of Pharmacy, Brooklyn, N.Y.

The present study sought to determine whether the endocrine and associated organ wts. as well as biochemical responses of histamine susceptible rats differed from those of histamine tolerant rats. Varying concentrations of aqueous histamine phosphate were injected intraperitoneally into male, Wistar rats. Rats which died from histamine shock were autopsied immediately. Rats surviving the 24 hrs. lethality observation period were then autopsied. Adrenal ascorbic acid in histamine susceptible and tolerant rats were also determined. The results showed that histamine tolerant rats had significantly lower thyroid, pituitary, spleen and liver wts., as well as markedly larger testes, prostate gland and adrenals. The tolerant animals had lower thymus wts. and significantly higher adrenal ascorbic acid levels. The ascorbic acid levels of the susceptible rats were inversely related to the histamine lethal dose. The adrenal, thymic, splenic, liver and ascorbic acid differences are in accord with the view of the physiodynamic endocrine balance.

Pharmacology

15

EFFECT OF RESERPINE ON ADRENAL CATECHOLAMINE AND ADENINE NUCLEOTIDES. W.R. Burack*, N. Weiner and P. Hagen, Dept. of Pharmacology, Harvard Med. School, Boston, Mass.

The adrenal gland contains an extraordinary concentration of adenine nucleotides (AN) in the same intracellular granules with stored catecholamines (C). The quantitative relation of AN to C in chicken adrenals was found to be severely altered following reserpine administration. AN were determined both chromatographically and enzymically and C colorimetrically in protein-free homogenate of whole gland. Control glands*(16) contained 17 μmol AN (58% ATP, 24% ADP and 18% AMP) and 66 μmol C/g wet weight. The "equivalence ratio," μmol C/total negative AN charges (assuming intragranular pH 7.0), was approximately unity. This by itself favors the concept that stored C uses AN for binding sites since each C molecule has a single positive charge. After 30 mg intramuscular reserpine (26 birds) there was profound depletion of both but more of C. Thus the "equivalence ratio" declined progressively for 3 weeks to 0.11. After 12 weeks it was still below control value. These results do not support the concept of inextricable ionic linkage. They suggest either that release involved an ion-exchange process or that some other anionic component shares in the binding complex and was also released. (Supported by USPHS grants B-1470, H-2205, HF-6523 and H-3674.)

XUM

16 *Pharmacology*

INHIBITORY ACTION OF ACETYL STROPHANTHIDIN ON THE SECRETION OF ALDOSTERONE. Allen W. Gomoll* and Theodore R. Sherrod. Dept. of Pharmacology, Univ. of Ill. Coll. of Med., Chicago, Ill.

A preliminary report on this topic (Pharmacologist, 1 #2: 63, 1959) suggested that acetyl-strophanthidin significantly decreased the secretion of aldosterone by the adrenal cortex in the intact anesthetized dog. Further investigation has substantiated these initial findings. Aldosterone analyses were carried out on blood samples taken before and after administration of a 1.2 mgm total dose of acetylstrophanthidin. Adrenal venous blood samples were obtained by cannulation of the right lumbo-adrenal vein in ether anesthetized dogs. The cortical steroids were extracted from the samples following the technique of Davis et al. (J. Clin. Invest. 36: 689, 1957) and silica gel chromatography. The aldosterone fraction was separated from other interfering steroids, Compounds B, E and F, by paper chromatography. The aldosterone was quantitated by the UV absorbtion of the aldosterone fraction at 239 mu and by the blue tetrazolium reaction. Administration of acetyl strophanthidin caused a significant decrease in the secretion of aldosterone by the adrenal cortex as well as a marked diminution in the rate of adrenal venous blood outflow. No consistant alteration in plasma Na, K, or Cl have been observed in these acute experiments incident to administration of acetyl strophanthidin. (Supported by Grant #H2354C2 from the Dept. of Health Education and Welfare, USPHS)

17 *Pharmacology*

THE UNUSUALLY POTENT ACTION OF THE CARCINOGEN 3-METHYLCHOLANTHRENE ON THE PITUITARY-ADRENAL SYSTEM IN THE RAT. W.R. Jondorf*, R.P. Maickel* and B.B. Brodie. Natl. Insts. of Health, Bethesda, Md.

Administration to male rats of 5 mg/kg I.P. of the carcinogen 3-methylcholanthrene (3-MCH) stimulated a sustained pituitary-adrenal response characterized by depletion of adrenal ascorbic acid, and elevated plasma levels of corticosterone and free fatty acids (FFA). Liver tryptophan peroxidase (TPO) activity was markedly increased, and serum alkaline phosphatase was substantially decreased. The levels of corticosterone and of FFA remained significantly elevated for 24-36 hrs and did not return to normal until 48 hrs. About 6 hrs after administration of the 3-MCH the animals were resistant to the sedative action of reserpine (2 mg/kg I.P.). This effect which lasted at least 24 hrs is similar to the antagonism of reserpine sedation by cold stress observed by Sulser and Brodie (this laboratory). A significant pituitary-adrenal stimulation lasting about 6 hrs could be observed with as little as 0.5 γ/kg of 3-MCH. None of the responses were elicited in hypophysectomized animals nor could an increased TPO be demonstrated in adrenalectomized animals. The results obtained with 3-MCH are of interest in view of the ability to increase ascorbic acid synthesis and activity of drug microsomal enzymes (Conney and Burns, Nature, 184: 363, 1959).

093. ENDOCRINES–Adrenal Cortex

Physiology

FUNCTIONAL AND ANATOMICAL INTEGRITY OF ADRENAL CORTICES NOT DEPENDENT UPON THE STRUCTURAL INTEGRITY OF THE HYPOTHALAMUS AND MIDBRAIN. D. M. Witt* and A. D. Keller. U.S. Army Medical Research Laboratory, Fort Knox, Kentucky.

Dogs were maintained for several weeks after midbrain and hypothalamic tissues were aspirated subsequent to section of the hypophysial stalk with scissors and thermocoagulation of the proximal stalk tissue. Thus, pars anterior and pars intermedia tissues remained insitu and were conclusively dissociated from neural or neurohumoral influences emanating from midbrain, hypothalamic or pars nervosal tissues. These preparations survived total pancreatectomy--performed two to four weeks after the hypothalamectomy and midbrainectomy procedure--without adenohypophysial or adrenal cortical supportive therapy. Pancreatectomy precipitated well defined diabetes mellitus. At autopsy the adrenal cortices were not atrophied and in instances exhibited varying degrees of hypertrophy.

2 *Physiology*

CORTICOSTEROIDOGENIC POTENTIAL OF REGENERATED RAT ADRENALS I. A. Macchi and Leland C. Wyman. Dept. of Biology, Boston Univ., Boston, Mass.

The corticosteroidogenic potential of regenerated rat adrenal autografts to mesenteric and intramuscular sites and of enucleated glands regenerated in situ was measured in vitro by blue tetrazolium and ultraviolet analysis of methylene dichloride extract residues of Krebs-Ringer incubation medium and in vivo by the water intoxication test. Compared to intact controls, adrenals regenerated 30 days or more exhibited normal in vitro output rates/unit tissue weight in the absence or presence of ACTHar (10 I.U./100 mg. adrenal) irrespective of regeneration site or age. Mesenteric transplants regenerated 14 days showed approximately 50% reduction in output rates without loss of ACTH sensitivity. None afforded normal protection against water intoxication after regenerating 30, 60 or 90 days. Enucleated glands were functionally superior in vivo at all periods to transplants while the mesenteric were superior at 60 but similar at 90 days to intramuscular transplants. Peak performance occurred at 60 days except with intramuscular transplants. Relative functional ability was generally but not consistently correlated with corresponding adrenal weight differences. Supported by grant C-3989, National Institutes of Health and ABC contract AT (30-1) 1254.

3 *Physiology*

ADRENOCORTICAL EFFECTS OF RESPIRATORY ACIDOSIS AND ITS COUNTERACTION WITH TRIS HYDROXY METHYL AMINO METHANE. A. Mittelman*, S. Dos*, G. Nahas, and G.H. Barker. Presbyterian Hosp. and Columbia U. Col. of Phy. and Surg., N.Y.

Anesthetized dogs were initially denitrogenated by mechanical ventilation following which "apneic oxygenation" was imposed for 1 hr. A recovery period on mechanical ventilation was then permitted followed by another hr. of apnea. During this 2nd apneic period 100 cc of 0.3 M tris hydroxy methyl amino methane (THAM) was given I.V. A 2nd recovery period on mechanical resp. followed. Thruout all phases of the experiments determinations of pH, HCO_3^-, pCO_2 and O_2 saturation were made on systemic arterial and venous and adrenal venous blood (left lumboadrenal V. catheter). Adrenal vein blood flows were measured as was the conc. of comp. F in adrenal V. blood. By the end of the 1st apneic period the arterial pH had fallen to 6.7-6.9 and pCO_2 was 150-300 but O_2 sat. remained normal. The recovery period restored normal pH and this was maintained during the 2nd apneic period with THAM. Adrenal blood flow was unchanged during the 1st apneic period and recovery but rose during THAM and then fell partially during recovery. Adrenal V. comp. F conc. rose during the 1st apneic period and remained high during recovery but fell during THAM and then rose again during recovery. (Supported by USPHS grant RG 2926-C8).

4 *Physiology*

ALDOSTERONE SECRETION FOLLOWING HEMORRHAGE IN HYPOPHYSECTOMIZED DOGS. P.J. Mulrow* and W.F. Ganong. Dept. Med., VA Hospital, West Haven and Yale Univ. School of Med., and Dept. Physiol., Univ. of Cal. School of Med., San Francisco, Calif.

Hemorrhage is known to stimulate the secretion of aldosterone. To determine the role of the pituitary in this response, 4 normal and 7 hypophysectomized dogs were acutely bled 15ml/Kg. body wgt. Control, post-hemorrhage, post-transfusion and post-ACTH samples of adrenal vein blood were collected in consecutive periods. In 4 normal and 6 hypophysectomized dogs, hemorrhage was followed by a rise in aldosterone secretion within 15 minutes which was sustained in all normal and 4 hypophysectomized dogs for at least 1 to 1 1/2 hours. In 2 normal dogs an associated increment in glucocorticoid secretion indicated an ACTH effect. Following transfusion of the shed blood, 3 of 4 normal but only 1 hypophysectomized dog showed a decrement in aldosterone secretion. One unit of ACTH administered intravenously resulted in a much greater aldosterone secretory rate than hemorrhage in the hypophysectomized and 3 normal dogs. It is concluded that hemorrhage in the hypophysectomized dog does stimulate aldosterone secretion but this secretory rate is less than that of the hemorrhaged normal dog or following ACTH administration. The data indicates that hemorrhage stimulates ACTH release.

5 *Biochemistry*

IDENTIFICATION IN HUMAN PLASMA OF NATURALLY OCCURRING 11-DEHYDROCORTICOSTERONE ACETATE. T.E.Weichselbaum and H.W.Margraf*. Washington University School of Medicine, St. Louis, Mo.

In attempting to isolate and identify adrenocortical steroids naturally occurring in human peripheral plasma, a compound was isolated from 2 different normal human plasma pools of 5 and 10 liters each which was identified as 11-dehydrocorticosterone acetate. This compound was found to be present in a concentration of approx. 3 gamma%. Behavior in various paper and column chromatographic systems, response to various chemical group reactions and micro-KBr-infrared spectroscopy have made a definite identification possible. Acetylation during the processes of isolation and purification has been experimentally excluded. Deacetylation by means of acetyl esterase isolated from orange flavedo resulted in the identification of 11-dehydrocorticosterone as the hydrolysis product. Due to the known properties of serum albumin to hydrolyze most steroid acetates promptly, except for this particular compound, it must be concluded that under appropriate conditions of blood collection other naturally occurring steroid acetates may be isolated and identified. The normal presence of C21 steroid acetates may well be of importance with respect to the transport mechanisms for steroid hormones. (Supported by a grant from the U.S. Public Health Service, C-2383(C4).

6 *Biochemistry*

DETERMINATION OF Δ 5-PREGNENE-3β,17α,20α-TRIOL (5PT) IN URINE. Hildegard Wilson, Natl. Insts. of Health, Bethesda, Md.

Current concepts of adrenocortical reactions presuppose that Δ^5-17-OH-pregnenolone (5-17PG) is the precursor of dehydroepiandrosterone (DHA) in the adrenal cortex. Since detection of 5PT, a C_{21} metabolite of 5-17PG, gives direct evidence for its synthesis, 5PT has been quantitatively determined in the urine of a variety of individuals. After separation of the 5PT fraction of urine extracts on a partition column, followed by digitonin precipitation and paper chromatography, specific assay was achieved by oxidation with HIO_4 which converts 5PT to DHA. Both steroids give characteristic absorption spectra, suitable for quantitation, with the Allen H_2SO_4 reagent. It was found that 5PT is a common but not invariable urinary constituent, and may be absent even when substantial amounts of DHA are excreted. Three persons without adrenocortical abnormality excreted about 55 µg per day, while two others had none. Five patients with adrenal carcinoma excreted increased amounts up to 15 mg., while in 3 subjects with Cushing's syndrome (1 with adrenal carcinoma), 5PT was absent, though DHA was present in 2. Thus, either 5-17PG is not a regularly synthesized precursor of DHA, or more probably, some subjects convert it completely and none is secreted unchanged.

7 *Biochemistry*

SURVEY OF THE PHOSPHODIESTERASE ACTIVITIES OF ADRENAL GLANDS. K. Arai and L. L. Salomon. (intr. by A. A. Ormsby) Dept. of Biochemistry and Nutrition, Univ. of Texas Med. Br., Galveston, Texas.

Preliminary studies on the distribution of the phosphodiesterase activities of adrenal glands (beef and rat) has shown the presence of four such activities in beef cortical tissue, characterized mainly by their particular pH optima in the presence of activators and inhibitors. Methods for assay, preparation of tissues and general properties of the enzymic activities, such as K_m's for various substrates, will be presented.

Supported by U.S.P.H.S. Grants A-2865 and C-3994.

8 *Biochemistry*

CONVERSION OF CHOLESTEROL TO PREGNENOLONE. George Constantopoulos* and T. T. Tchen, Dept. of Chem., Wayne State University, Detroit 2, Michigan.

The steps involved in the conversion of cholesterol to pregnenolone by adrenal cortical mitrochondria are being investigated. In order to follow both fragments formed by the cleavage of the side chain, cholesterol labelled both in the rings and in the side chain is used. It has been shown that when TPNH is used as the only cofactor, polar sterol(s) is formed, but no labelled CO_2 or steam-volatile acids can be detected. It has also been shown that the formation of labelled CO_2 requires coenzyme A and probably lipoic acid. The nature of this polar intermediate and the overall mechanism of the cholesterol-pregnenolone conversion will be discussed. (Supported by grants from Research Corporation, Life Insurance Medical Research, United States Public Health.)

9 *Pharmacology*

THE ACTIVATION OF ADRENAL PHOSPHORYLASE BY CYCLIC 3',5'-ADENYLATE. Gene A. Riley and Robert C. Haynes, Jr. (intr. by E. W. Sutherland). Western Reserve Univ. School of Med., Cleveland, O.

Soluble extracts of beef adrenal glands respond to the addition of ATP and Mg^{++} by an activation of phosphorylase. When cyclic 3',5'-adenylate (3,5-AMP) is also added, the amount of activation is increased. The preparations retain this capacity to respond to 3,5-AMP after precipitation at pH 5.4, or ammonium sulfate or alcohol precipitation and passage over diethylaminoethyl cellulose and carboxymethyl cellulose columns. Activity remains after dialysis. This phosphorylase-activating system appears to differ in several ways from comparable systems prepared from dog liver and rabbit skeletal muscle. Thus the activation of adrenal phosphorylase is not stimulated by zinc ions, and it does not show the sharp pH dependence reported for skeletal muscle preparations. It is hoped that future studies will indicate whether the effect of 3,5-AMP is on an enzyme system that controls the activity of the phosphorylase kinase or on a non-enzymatic inhibitor of the kinase.

10 *Pharmacology*

EFFECT OF CORTISOL AND DEOXYCORTICOSTERONE (DCA) ON ELECTROSHOCK SEIZURE PATTERNS IN DEVELOPING RATS. Antonia Vernadakis* and Dixon M. Woodbury. Dept. of Pharmacology, Univ. of Utah, Salt Lake City, Utah.

Electroshock seizure patterns in developing rats were studied by Millichap (Proc. Soc. Exper. Biol. & Med. 96: 125, 1957) and confirmed in our laboratory. The maximal seizure patterns obtained are as follows: newborn to 8-day-old rats displayed only hyperkinesis; 8 to 17-day-old rats exhibited a clonic seizure; 17 to 45-day-old rats displayed the adult tonic pattern. Chronic cortisol administration (10 mg/kg/day) induced the following changes in seizure patterns: hyperkinesis was increased in 8-day-old rats; latency was shortened and clonus was more severe in 12-day-old rats; flexion decreased and extension increased in 17- and 21-day-old rats. In contrast to cortisol, DCA administration (10 mg/kg/day) did not alter seizure patterns in 8-, 17-, and 21-day-old rats.However, DCA did increase latency and eliminate clonus in 12-day-old rats. The observations suggest that cortisol induces an 'aging' effect on the development of seizure patterns, whereas DCA induces a retarding effect. (Supported by Grant B-381, N.I.H. and Nat'l. Neurological Research Found.)

095. ENDOCRINES–Adrenal Cortex–Stress

Physiology

ADRENALINE, NORADRENALINE, AND 3-METHOXY-4-HYDROXY-MANDELIC ACID (MOMA) EXCRETION FOLLOWING CENTRIFUGATION AND ANTICIPATION OF CENTRIFUGATION. Lawrence Berman and McC. Goodall (Intr. by Horace O. Parrack), Acceleration Sec. Biophysics Br. Aerospace Medical Lab. Wright-Patterson AFB, Ohio, and Research Center and Hospital, Univ. of Tenn. Knoxville, Tenn.

The purpose of these experiments was to determine the effect of centrifugation (gravitational stress) and the anticipation of being centrifuged upon the release of adrenaline, noradrenaline, and their common metabolic product, MOMA. Nine Air Force subjects were centrifuged at 1g/5 sec to 12g or were given a "mock" ride. Each subject was unaware as to whether he would receive a real ride or a "mock" ride. Therefore it was possible to measure the sympatho-adrenal response to both centrifugation and anticipation of centrifugation. Urine samples were collected before and after each ride and bioassayed for adrenaline and noradrenaline; the MOMA was determined chromatographically. Both centrifuged subjects and those given the mock ride show activation of the sympatho-adrenal system such that there is an increase in the output of adrenaline and noradrenaline plus a concomitant rise in MOMA excretion.

2 *Physiology*

SURVIVAL OF ADRENALECTOMIZED ANIMALS EXPOSED TO LOW BAROMETRIC PRESSURE. D. A. DeBias and K. E. Paschkis (intr. by J. M. Waldron.) Dept. of Physiology, Jefferson Med. Coll., Phila., Pa.

Recent studies have shown that certain factors involved in survival under a particular stressful situation are specific for that situation. In experiments reported here the survival rate of adrenalectomized animals pretreated with neuropharmacological agents and exposed to low barometric pressure (249 mm Hg; simulated altitude of 27,000 ft.) was compared to that of untreated animals similarly stressed. The survival rates were also compared to those of animals similarly treated and exposed to formalin injection, to cold exposure (2-4° C), and to heat exposure (37.5° 38.5° C). Whereas Dibenamine and chlorpromazine were protective against formalin injection, Pendiomide ditartrate against cold exposure, and Pendiomide and chlorpromazine against heat exposure, none of the agents tested exerted any beneficial effect on the survival rate of animals exposed to low barometric pressure. Doses of cortisol which did not protect adrenalectomized animals against low barometric pressure became highly effective when the adrenalectomized animals were also thyroidectomized. (Supported by USAF Contract AF 41(657)-253).

3 *Biochemistry*

PLASMA LEVELS OF CORTICOSTERONE AFTER ADRENAL ENUCLEATION P.M. Hyde, and F. R. Skelton.* L.S.U. School of Med. and Urban Haas Res. Fdn., New Orleans, La.

In the study of adrenocortical function in relation to adrenal regeneration hypertension, the capacity of the glands to respond to stress was determined in rats with 1) both adrenals intact, 2) two regenerating adrenals (RA) and 3) one regenerating adrenal. Groups of 20 such animals were given water or saline to drink, or uninephrectomy and saline. The rats were decapitated under "resting" conditions or 1 hour after bilateral fracture of tibia and fibula, 21 days after enucleation. Corticosterone in plasma pools was measured by isotopic dilution techniques using corticosterone-4-C^{14} and the procedure of Zenker and Bernstein (J. Biol. Chem. 231, 695 (1958).

		μg. Corticosterone per 100 ml. Plasma.		
		Water	Saline	Saline & Nephr.
Intact Adr.	Unstressed	5.2	4.6	5.1
	Stressed	61.5	69.2	41.3
Double RA	Unstressed	6.6	5.3	4.7
	Stressed	41.0	29.8	37.4
Single RA	Unstressed	7.1	3.8	3.2
	Stressed	26.7	38.0	30.2

Rats with regenerating adrenals seem less capable of responding to stress than controls. Supported by Grants from USPHS (H-3193) and Edward G. Schlieder Fdn.

4 *Biochemistry*

EFFECT OF SPLEENECTOMY ON THE ADRENAL AND PLASMA CORTICOIDS OF FASTED RATS. Grant G. Slater (intr. by R. B. Alfin-Slater). Depts. of Psychiatry and Physiological Chemistry, School of Medicine, Univ. of California Med. Ctr., Los Angeles, Cal.

Earlier studies (Fed. Proc. 18; 325, 1959) demonstrated that following unilateral adrenalectomy of young fasted rats, no decrease was produced one hour later in the contralateral adrenal ascorbic acid (AAA). In these studies 80 gm. rats were fasted 3 days, anesthetized with Nembutal, and 15 min. after injection were subjected to the acute stress of spleenectomy. After 15, 30, & 60 min. the AAA, left adrenal, adrenal corticoids (AC), right adrenal, and plasma corticoids (PC) were determined. At 15, 30, and 60 min. the AAA values were 5%, 13% & 13% respectively, below fasted controls; concomitantly the AC were 44%, 585% & 500% and the PC were 72%, 189% & 283% above fasted controls. The AC 15 min. after stress were high in rats with high AAA, but at 30 and 60 min. the highest AC were obtained from rats with the lowest AAA. The PC were lowest in rats with the highest AAA. The PC in rats with low AAA showed a decrease in PC between 15 and 30 min.; however, PC increased during this time in rats with high AC. (Supported by a grant from California State Dept. Mental Hygiene.)

5 *Biochemistry*

ADRENOCORTICAL RESPONSE OF NEWBORN RATS TO STRESS AND VASOPRESSIN. Shawn Schapiro (intr. Joseph Katz.) Vet. Adm. Center, Los Angeles and Med. Center, Univ. of Calif., Los Angeles.

The adrenal ascorbic acid (AAA) depletion occuring one hour after electric shock stress in infant rats was measured. The ages of the animals ranged from 1 day to 30 days after delivery. The AAA response to commercial pitressin, lysine vasopressin (Guillemin, et al., Proc. Soc. Exp. Biol. & Med.: 101, 107, 1959) and ACTH was also determined in 1-10 day old animals.

The adrenal of the infant rat did not respond to shock or surgery with an AAA depletion. A marked decrease was observed with 20 milliunits of ACTH, IP. 50 milliunits commercial vasopressin, IP, elicited a decrease in AAA in the 1-10 day old rat while Guillemin's lysine vasopressin in equivalent doses was followed by only insignificant changes.

It is suggested that, because the newborn rat does not respond to stress it may serve as a test animal for hypothalamic neurohumoral agents implicated in the release of ACTH.

6 *Pathology*

SUPPRESSION OF STEROID DIABETES IN RATS BY EXPOSURE TO STRESS. David J. Ingle (intr. by Dwight J. Ingle.) The Ben May Laboratory for Cancer Research, The University of Chicago, Chicago, Illinois.

This report relates to an earlier study showing that the glycosuria of rats having steroid diabetes is suppressed either by implantation of Walker Carcinoma 256 or by the excision of skin. Young adult male rats of the Sprague-Dawley strain were tube-fed a medium carbohydrate diet twice daily and each received 10 mg. of cortisone acetate daily for 10 days. All of the rats developed severe glycosuria. Twenty-two rats were kept under non-stress conditions. Twelve rats stressed by the injection of 0.5 cc of 1.5 % formaldehyde/day showed a significant suppression of glycosuria and 11 rats stressed by restriction of activity showed a marked suppression of glycosuria below that of non-stressed controls. From this and earlier studies it is concluded that at least some stressors can suppress at least one secondary symptom (glycosuria) of hypercorticalism in the rat. (Supported by USPH Grant A-1653 and by a grant from Eli Lilly and Company.)

096. ENDOCRINES–Adrenal Medulla

Biochemistry

ESTIMATIONS OF URINARY MANDELIC ACID DERIVATIVES AND CATECHOLAMINES IN THE DIAGNOSIS OF PHEOCHROMOCYTOMA. F. William Sunderman, Jr.,* Philip D. Cleveland,* and F. William Sunderman. U.S. Naval Medical School, Bethesda, Maryland and Jefferson Medical College, Philadelphia, Pa.

Mandelic acid derivatives and catecholamines have been measured in urine samples from normal individuals and hypertensive patients suspected of suffering from pheochromocytoma. The catecholamine excretion in 47 normal individuals ranged from 9 to 102 mcgm per 24 hours (mean 46, S.D. ± 22). The excretion of mandelic acid derivatives in 30 normal individuals ranged from 0.7 to 6.8 mg per 24 hours (mean 3.9, S.D. ± 1.6) In five patients with pheochromocytoma the catecholamine excretion ranged from 225 to 4500 mcgm per 24 hours, and the excretion of mandelic acid derivatives ranged from 15 to 250 mg per 24 hours. Comparisons are made of diurnal fluctuations in the excretion of these components and of their relative precision and adaptability to routine measurements. In the authors' experience the estimation of urinary mandelic acid derivatives has several advantages over the estimation of urinary catecholamines as an aid in the diagnosis of pheochromocytoma. (Supported in part by grant to Jefferson Medical College from the Atomic Energy Commission.)

2 *Pharmacology*

STUDIES ON THE POSSIBLE RAPID BIOSYNTHESIS OF NOR-ADRENALINE FROM INFUSED DIHYDROXY PHENYLETHYLAMINE (DOPA-MINE) IN ANESTHETIZED DOGS. Walter M. Booker, Delores Edwards*, Estela Gonsales*, and Mabel Robinson*. Dept. of Pharm., Howard Univ. Med. Sch., Wash., D. C.

The results so far indicate a marked increase in positive inotropic action of the heart from plasma samples (crude and extracts) collected during infusion of DOPA-mine above that of the control collection. Crude plasma samples and aluminum oxide extracts of plasma are introduced into the isolated perfused guniea pig heart (Langendorff technique) and the positive inotropic effect on the heart noted and compared with standards of DOPA-mine and of nor-adrenaline. The increase in inotropic action is so greatly in excess of that of DOPA-mine standards and in line with those of nor-adrenaline that we believe DOPA-mine may be synthesized to nor-adrenaline in a matter of a few minutes. Paper chromatographic analysis is being made of each plasma sample and of DOPA-mine and nor-adrenaline standards, comparing the RF values, in order to further elucidate this problem.

3 *Pharmacology*

FURTHER STUDIES ON THE ESTIMATION OF INFUSED ADRENALIN AND NOR-ADRENALIN, USING THE ISOLATED PERFUSED GUINEA PIG HEART. A. F. DeSchaepdryver*, Walter M. Booker, T. Walton*, E. Fisher* and R. Linares*. Dept. of Pharm., Howard Univ. Med. Sch., Wash., D. C.

Previous experiments have indicated a high sensitivity of the isolated perfused guinea pig heart to catechol-amines in plasma, when crude plasma samples or aluminum oxide extracts are introduced into the system (Langendorff technique) following infusion of catechol-amines into anesthetized dogs. The present experiments were undertaken to confirm preliminary observations and to determine if certain amine-oxidase inhibitors or other enzyme inhibitors (transferase) might influence appreciably the plasma concentration of adrenaline or nor-adrenaline upon constant infusion of these catechol-amines. Experiments indicate that higher concentrations of plasma nor-adrenaline are detectable following 30 minute infusion of nor-adrenaline than may be detectable following constant infusion of adrenaline for 30 minutes. Both substances are infused at 1.5 gamma/kg/min. J. B. 516 or/and pyrogallol, in the experiments so far, have not greatly altered the concentration of these catechol-amines following constant infusion. Other areas of disappearance of these catechol-amines are being searched for now.

097. ENDOCRINES–Anterior Pituitary

Physiology

STIMULATION OF AMINO ACID TRANSPORT IN MUSCLE BY OXYCEL ACTH IN VITRO. J.L.Kostyo* and F.L.Engel. Duke Univ., Durham, N.C.

ACTH, like STH, decreases urea formation in adrenalectomized-nephrectomized rats. To examine further the extra-adrenal action of ACTH in nitrogen metabolism, its effect on in vitro transport of α-aminoisobutyric acid-1-C^{14} in rib cage preparations of hypophysectomized rat diaphragms was studied. AIB-1-C^{14} transport was increased two-fold by bovine STH in vitro (1 µg/ml medium). Oxycel ACTH (25-100 µg/ml) produced a similar increase, but 5 µg/ml was inactive. Preparations of α_1-α_2 corticotropin (Bell), corticotropin A_1 and periodate borohydride-treated corticotropin A_1 (Dixon) had no effects at concentrations of 25 µg/ml. Other concentrations were not tested. Since, in contrast to STH, oxycel ACTH stimulated transport at high concentrations and more purified corticotropins were inactive at similar concentrations, the observed effect may be due to STH contamination. Treatment with 0.1 N NaOH at 25°C abolishes STH but not ACTH activity on urea formation. However, this treatment did not destroy the transport effect of STH and hence could not be used to eliminate contamination of oxycel ACTH with STH. Treatment of oxycel ACTH with H_2O_2 abolishes its activity on urea formation but has yielded equivocal results, so far, on its transport activity. Further studies are underway to determine if the transport activity represents an extra-adrenal property of corticotropin or is due to contamination.

2 *Physiology*

THE BIOLOGICAL HALF-LIFE OF L.H., P.M.S., AND H.C.G. IN THE RAT. Albert F. Parlow (intr. by L.P. Wilson) Vincent Memorial Hospital and Harvard Medical School, Boston, Mass.

The rate of disappearance of P.M.S., H.C.G. and both heterologous (ovine) and species-specific L.H. from the plasma of the rat following a single intravenous injection has been determined. Donor rats were adult females weighing 210-270 gm. They were injected intravenously with either a) P.M.S., 500 I.U. per 100 gm., b) H.C.G., 250 I.U. per 100 gm. c) ovine L.H., the equivalent of 50 micrograms of NIH-LH-S-1 or d) murine (rat) L.H., the equivalent of 25 micrograms of NIH-LH-S-1. Groups of 5 rats each were bled at 3 time intervals following intravenous injection: a) P.M.S., 1,24,72 hours b) H.C.G., 3,6,12 hours c) ovine L.H., 10,20,40 minutes d) murine L.H., 5,18,44 minutes. Plasma was bioassayed for P.M.S., H.C.G. or L.H. activity by the ovarian ascorbic acid depletion method (Parlow, Endocrinology, in press, 1960). The mean biological half-lives were: a) P.M.S., 26 hours (2 determinations) b) H.C.G., 4.9 hours (3 determinations) c) ovine L.H., 15 minutes (1 determination) d) murine L.H., 17 minutes (1 determination). Each gonadotropin can be characterized by the slope (in logs) of its disappearance curve (log concentration v. time). The values are a) P.M.S. - 0.012 b) H.C.G. - 0.064, c) ovine L.H., - 1.34 d) murine L.H. -1.07. Ovine L.H. was not excreted.

3 *Pharmacology*

DIRECT MEASUREMENT OF ESTROGENS (Es) IN THE OVARY (O) AND OVARIAN VENOUS BLOOD (OVB) IN THE RAT AS AN ASSAY FOR LH C. Johnson, R. Franklin, V. Critchlow, H. Lipscomb, G. Clayton and R. Guillemin (intr. by W. Krivoy). Dept. of Physiology, Baylor U. Coll. of Med., Houston, Texas.

Rats (200g BW) were "primed" with PMS and HCG (Parlow, Fed. Proc. 17:402, 1958). Ovaries were extracted with alcohol and benzene and the residue chromatographed on paper in a hexane-benzene-formamide system. Residues of similarly extracted OVB from animals prepared as above were chromatographed in the same manner. Areas on chromatograms corresponding to those of simultaneously run standards of estrone, estradiol 17β (E2) and estriol (E3) were eluted and quantitative measurements carried out by H_2SO_4-induced fluoresence on eluates. Total Es were measured by fluorometry on residues extracted from both O and OVB. Injection of LH (Armour Std. 227-80) in jugular vein of "primed" rats increases the total amount of fluorescent material extracted as above from O and OVB and also alters the ratio of fluorescent materials from the E3/E2 paper areas from residues of both O and OVB, as early as 60 min. after injection. Using total fluoresence on extracts of both O and OVB, a log dose response to i.v. injected LH was observed between 0.25 and 4.0 µgm.

XUM

4 *Pathology*

THE EFFECT OF CIRRHOSIS OF THE LIVER ON GONADOTROPIN CONTENT OF THE HUMAN HYPOPHYSIS. Agnes B. Russfield and S. C. Sommers. Bio-Research Inst., Inc., Cambridge, and Mass. Mem. Hosps., Boston, Mass.

On the basis of histopathological studies, it has been suggested that patients with cirrhosis of the liver, particularly when accompanied by hepatoma, may have increased hypophyseal gonadotropins (Barr and Sommers, J. Clin. Endocrinol. & Metab. 17:1017, 1957). In order to test this theory, hypophyses were removed at autopsy from 12 cirrhotic patients and assayed for gonadotropin content in immature hypophysectomized female rats. Results were compared with assays on patients of comparable ages dying of other diseases. The hypophyses of 5 of the 8 men contained unusually large amounts of stored gonadotropins. Two of these patients had hepatomas and 3 had uncomplicated cirrhosis. Stored gonadotropins were in the normal range in 2 cirrhotic men and were greatly reduced in one who had received stilbestrol for an incidental carcinoma of the prostate. Stored gonadotropins were in the normal range in 3 females and were reduced in one who had received Meticorten therapy. It was concluded that there is good biological evidence for increased gonadotropin storage in some male cirrhotics.

5 *Pathology*

CHARACTER OF PITUITARY TUMORS INDUCED IN RATS BY HEAD-NECK X-IRRADIATION. Kenjiro Yokoro* and Jacob Furth. Roswell Park Mem. Inst., Buffalo, N.Y.

Pituitary tumors were induced in mice by whole body irradiation(Radiol., 63:562,1954)., in rats by localized deuteron irradiation (Endocrinol.,64:240, 1959). The mouse tumors were adrenotropic or mammotropic. The type of rat tumor is unknown. Of 27 WR 1 month old female rats given 800r x-ray over head-neck, 70.4% developed pit. tumors within 2 years (mean 21 months). In contrast, 13.7% of normal female rats had tumors within 2 years, and 27.3% at mean age of 23 months (J.N.C.I., in press). Tumor bearing hosts revealed marked mammary hyperplasia with secretion and adenomatoid lesions. This was confirmed by transplantations. To date 2 pit. tumors were transplanted with success on WR rats. Both proved to be highly mammotropic. The other endocrine organs were not stimulated. 3 mammary and 2 other tumors (18%) occurred in the radiation field, none elsewhere. These experiments indicate that head-neck irradiation markedly increases the incidence of mammotropic pit. tumors, accelerates their development and produces other tumors in the radiation field. (Supported by grants from AEC and NCI)

098. ENDOCRINES–Anterior Pituitary–ACTH

1 *Biochemistry*

A SIMPLIFIED METHOD FOR THE PREPARATION OF ACTH. L. L. Rosenberg and E. S. Evans.* Inst. Exper. Biol. and Dept. of Anat., Univ. of Calif., Berkeley.

The brief method for the preparation of sheep ACTH herein described grew out of the study of erythropoietic factors of the pituitary. Four kg. of ground sheep pituitaries were extracted at 3°C with 0.1N HCl; the mixture was adjusted to pH 5.5 and heated over a flame to 85°C. The material was filtered and acetic acid was added to the clear filtrate to give a concentration of 0.1M. ACTH was adsorbed onto oxycellulose and then eluted with 0.1M HCl. The eluate adjusted to pH 7 was lyophilized (to reduce the volume) and dialyzed to remove salt. The product was redried and dissolved in 0.05M $NaHCO_3$ and put onto an IRC-50 column which had been equilibrated with 0.05M $NaHCO_3$. The unadsorbed material was discarded. The column was washed with 0.05M $NaHCO_3$ and then the ACTH was obtained by gradient elution between 0.05 and 0.15M $NaHCO_3$. The ACTH eluate adjusted to pH 6.5 was lyophilized, dialyzed, and then re-lyophilized. The procedure is reproducible and consistently gives a product in good yield with high potency. This material is a potent stimulant of calorigenesis and erythropoiesis in hypophysectomized rats.

2 *Pharmacology*

STUDIES ON EXTRACTION OF BLOOD MELANOPHORE HORMONE ACTIVITY. Robert S. Teague and Jane Reid Patton*. Dept. of Pharmacol., Univ. of Ala. Med. Centr., Birmingham, Ala.

Our current need is a procedure which will yield a fraction from blood containing all the melanophore hormone activity, in a concentrated, partly purified form suitable for assay by our reflection technique. As a preliminary, it was necessary to determine whether activity was carried by the blood cells, plasma, or both. Samples of concentrated USP Posterior Pituitary Reference Standard were added to heparinized dog blood before and after separation of either serum or plasma from the cells. After a period of equilibration, the whole blood was processed and assayed. The results indicated the activity could be found in plasma and serum and not in the cells. Recovery extraction studies were then begun with rat serum, which had been enriched by homogenizing fresh, whole rat pituitaries in the serum. Aliquots were lyophylized, homogenized at 79° C. with glacial acetic acid, centrifuged, and the supernatant lyophylized. These dry solids have been treated in two ways: 1) extraction with a small amount of glacial acetic acid, dilution and neutralization; 2) extraction with methanol three times, concentration, and dilution with water to about 20% methanol. Assays of 1) and 2) showed that the latter yielded only 60% of the activity of the former. (Supported by USPHS Grant 3101-C.)

099. ENDOCRINES–Anterior Pituitary–Control

Physiology

RELEASE OF PROLACTIN FROM THE ANTERIOR PITUITARY BY NEUROHORMONES, DRUGS, HYPOTHALAMIC TISSUE AND OTHER AGENTS. Joseph Meites and T. F. Hopkins*. Dept. of Physiology and Pharm., Michigan State University, East Lansing, Mich.

Numerous agents have been tested in our laboratory in an attempt to elucidate the mechanisms controlling the release of prolactin and possibly other AP hormones which promote mammary growth and lactation. Intact mature female rats were injected with estradiol for 10 days to partially develop their mammary glands, and were then treated with one of the test agents for 5 days. Mammary glands were removed on the 16th day for histological examination. The following agents induced mammary growth and lactation: epinephrine, norepinephrine, acetylcholine, serotonin, pilocarpine, physostigmine, atropine, reserpine, chlorpromazine, meprobamate, amphetamine, morphine, 3-methylcholanthrene, benzpyrene, DMBA, stimulation of uterine cervix, several nonspecific stresses, and hypothalamic tissue. Oxytocin, vasopressin, histamine, dibenemine, LSD-25, kidney and liver were ineffective in the doses given. Epinephrine, acetylcholine and serotonin induced mammary growth and gross lactation in estrogen-primed female rabbits, but were ineffective when injected into hypophysectomized rats. These results suggest that release of prolactin and possibly other AP hormones favorable to mammary growth and lactation is mediated through the autonomic nervous system and hypothalamus (Supported by NIH grant C-3448).

2 *Physiology*

INITIATION OF LACTATION IN RATS BY NONSPECIFIC STRESSES. Charles S. Nicoll (intr. by B. V. Alfredson). Dept. of Physiology and Pharmacology, Michigan State University, East Lansing.

In order to investigate the effects of nonspecific stresses on the secretion of prolactin from the anterior pituitary, 60 mature virgin female rats were divided into 6 groups of 10 each and injected for 10 days with 10 ugm estradiol daily. For the following 5 days the groups were treated as follows: 1) controls, saline injected once daily; 2) cold (0°C) 24 hrs./day; 3) intense heat and light, 12 hrs./day; 4) restraint, 12 hrs./day; 5) starvation; 6) injection of 10% formaldehyde once daily. On the 16th day the rats were killed and the mammary glands were removed for histological examination. The mammary glands of the control rats regressed from a lobuloalveolar system to bare ducts during the period of saline injections. Variable degrees of lactation were induced in all the stressed groups as indicated by secretion in the alveoli. It is concluded that nonspecific stresses can promote secretion of prolactin as well as ACTH from the anterior pituitary in amounts sufficient to induce lactation in estrogen primed rats. A common mechanism regulating the secretion of ACTH and prolactin is considered. (Supported by NIH grant C-3448 to J. Meites.)

3 *Physiology*

INABILITY OF THE PITUITARY HOMOTRANSPLANTED TO THE KIDNEY CAPSULE TO SECRETE ACTH IN MALE RATS. R. Foster, Jr.* and I. Rothchild. Dept. Obstetrics and Gynecology, Western Reserve U., Cleveland.

Ten-week old male rats bearing pituitary homotransplants (APtr) beneath the kidney capsule (transplanted 3 weeks previously) were hypophysectomized 24 hours before testing the transplant's ability to release ACTH. The results are expressed as the mean of the difference in adrenal ascorbic acid concentration between the left adrenal, removed at the start of testing, and the right adrenal, removed one hour later(mg. ascorbic acid/g. adrenal). (Value in parentheses= Standard Deviation). The results after ether anesthesia and left adrenalectomy were: 7 intact rats without APtr: 197(20); 8 hypophysectomized, without APtr: 4(36); 10 sham hypophysectomized, without APtr: 84(44); 10 sham hypophysectomized, with APtr: 90(71); 17 hypophysectomized with APtr: 20(42). Intravenous histamine (1.0 mg./100 g. body wt.) plus the above stimuli gave the following results: 7 intact without APtr: 191(26); 7 hypophysectomized, without APtr: -13(33); 11 sham hypophysectomized, without APtr: 112(67); 7 sham hypophysectomized with APtr: 103(68); 13 hypophysectomized with APtr: -7(43). The results suggest that the transplant is unable either to form ACTH or to respond to the factors that cause ACTH release from the pituitary in situ following such stimuli.

4 *Nutrition*

INITIATION AND MAINTENANCE OF MILK SECRETION IN THE RAT FOLLOWING CHLORPROMAZINE ADMINISTRATION. P. K. Talwalker (intr. by C. A. Hoppert). Dept. of Physiology and Pharmacology, Michigan State University, East Lansing.

Chlorpromazine has been shown to induce pseudopregnancy and also cause release of ACTH from the AP in mature female rats. It was of interest to determine whether it would also induce release of prolactin in amounts sufficient to initiate or maintain lactation. The mammary glands of mature female rats were developed by injecting 10 ug estradiol daily for 10 days, and subsequently chlorpromazine was injected daily for 5 days at dose levels of 5 or 15 mg/kg body weight. On the 16th day the mammary glands were examined histologically. Chlorpromazine induced secretion only in the rats given the higher dose. In another experiment, chlorpromazine at dose levels of 5 or 15 mg/kg body weight was injected daily for 10 days in postpartum rats after removal of their litters on the 4th day. Twenty-four hours after the last injection, the mammary glands were examined histologically. At the higher dose level, chlorpromazine inhibited mammary involution and maintained secretion. It is concluded that chlorpromazine induces release of prolactin and possibly other AP hormones in amounts adequate to initiate and maintain lactation in the rat. (Supported by NIH grant C-3448 to J. Meites).

100. ENDOCRINES–Anterior Pituitary–Somatotrophin

Physiology

NUCLEI PER MM^3 OF SKELETAL MUSCLE IN NORMAL ADULT AND PITUITARY GIANT FEMALE RATS. J. D. Emerson and G. M. Emerson.* Dept. of Physiol., Univ. of Ala. Med. Center, Birmingham.

Adult Norway rats treated with anterior pituitary growth hormone, or growth hormone containing extracts, eventually cease growing at a given dosage per unit weight irrespective of how great this dosage. This phenomenon (plateauing) appears not to result from diabetes, circulating antibodies, aging of the rats, alteration of cardiac index, steroid metabolism or metabolic rate. To determine if plateauing could be due to a decrease in nuclei/cytoplasm, we have measured maximum diameter and nuclei per 10 u length of 1600 randomly selected gastrocnemius fibers from 8 plateaued giant and 8 normal adult female rats.

	Pit. Giant	Normal Adult	17 Day Infant
Body Wt. (gm.)	631.4	266.9	29.7
No. of Fibers	1600	1600	1200
M Nuclei/10u ln,	4.47±0.05*	3.32±0.04	1.81±0.03
Nuclei/mm^3 x 10^4**	21.23±0.36	17.10±0.28	167.89±2.42

*Std. error of mean. **Area of fiber calculated from diameter. Aided by a grant from Muscular Dystrophy Associations of America, Inc.

2 *Biochemistry*

PHYSICOCHEMICAL AND IMMUNOCHEMICAL CHARACTERIZATION OF SWINE PITUITARY GROWTH HORMONE. Harold Papkoff*, Raghuveer N. Moudgal*, and Choh Hao Li. The Hormone Research Laboratory University of Calif., Berkeley, Calif.

Purification of growth hormone from pig pituitaries has been reported by Raben, Wilhelmi, Ottoway and others. Using a procedure similar to that used for the preparation of growth hormone from other species, we have isolated the swine growth hormone in highly purified form. In buffers of 0.1 μ, the preparation was isoelectric at pH 6.3. In borate buffers of pH 9.9, the hormone had $s_{20,w}$ of 3.02S, and a diffusion coefficient of 6.54 cm^2/sec. Assuming $\bar{v}$ to be 0.73 ml./gm., a molecular weight of 41,600 was calculated. Spectrophotometric analysis indicated 5.59% tyrosine and 1.38% tryptophan, and a preliminary amino acid analysis indicated the presence of 3 residues of cystine. The FDNB procedure showed phenylalanine to be the N-terminal amino acid; studies with carboxypeptidase also identified phenylalanine at the C-terminus. Digestion with chymotrypsin to the extent of 25% non-protein nitrogen could be achieved without loss of biological activity. The pig hormone was also characterized immunologically by the quantitative precipitin test with rabbit antiserum. It was further shown that the antiserum to the pig hormone reacted with whale but not with the human growth hormone. This work is supported in part by the American Cancer Society.

3 *Biochemistry*

EFFECTS OF GROWTH HORMONE ON INCORPORATION AND TRANSFER OF N^{15} FROM AMMONIUM CITRATE. Trieste Vitti* and O. H. Gaebler. Edsel B. Ford Inst. for Med. Res., Henry Ford Hosp., Detroit.

Procedures developed in preceding studies with four N^{15}-labeled amino acids (Arch. Biochem. Biophys. 84, 188, 1959) were used. Seven untreated hypophysectomized rats, and an equal number treated daily for 5 days with 100 μg. intraperitoneal injections of growth hormone, received N^{15} (0.127 mEq 100 gm body weight) in the form of intraperitoneally injected ammonium citrate. Growth hormone increased incorporation of N^{15} from this source into TCA precipitated protein of liver, heart, kidney, and spleen, and into the sarcoplasmic, myofibrillar, and connective tissue fractions of muscle. TCA precipitated liver protein was hydrolyzed. From the hydrolysate, 9 amino acids and ammonia from amide nitrogen were isolated by column chromatography and excess of N^{15} determined. Threonine was not labeled. Labeling of other amino acids and ammonia, in atoms per cent excess, for untreated and treated groups was: phenylalanine .006, .006; tyrosine .011, .010; glycine .011, .013; serine .010, .014; arginine .033, .046; glutamic acid .033, .053; aspartic acid, .024, .038; alanine .034, .054; ammonia .057, .071. Thus incorporation of N^{15} into the last 5 compounds, to which considerable amounts were transferred in the untreated animals, was increased by growth hormone, but the transfer pattern was not altered.

4 *Pathology*

HUMAN SOMATOTROPIN ACTIVITY IN TISSUE CULTURE. Henry D.Moon, and Virginia L.Jentoft*,Dept.of Pathology,Univ.of Calif.School of Medicine,San Francisco,California.

The effect of human somatotropin on human cells was studied in tissue culture. A strain of cells,derived from liver (Chang), was grown in T-flasks using Medium 199 with 10% horse serum; the initial concentration of cells was 100,000/ml. After incubation for 24 hrs.at 37°C the tyrosine content of the cells and number of nuclei were determined to establish the baseline. The culture medium of the remaining flasks was replaced by the experimental and control media. The experimental medium contained 150mcg/ml human growth hormone. Human serum albumin 1500mcg/ml,ovine FSH 150mcg/ml,and Medium 199 alone were used as controls. The cultures were incubated for 72 hrs.at 37°C and the experiment terminated. The culture medium was removed and the T-flasks rinsed with salt solution. The number of nuclei and tyrosine content were determined in the experimental and control groups. The number of nuclei in the experimental group was greatly increased over the controls. The tyrosine content of the cells grown in human somatotropin was also increased over the several controls. These data indicate that human somatotropin promotes nuclear multiplication and protein synthesis of human cells in tissue culture. (Supported by USPHS Grant CY3341 and ACS Grant P-113).

XUM

101. ENDOCRINES–Glucocorticoids

Biochemistry

EXTENSION OF THE PORTER-SILBER REACTION TO α-KETOLIC STEROIDS LACKING A HYDROXYL GROUP AT C-17. Marvin L. Lewbart* and Vernon R. Mattox. Mayo Found. and Mayo Clin., Rochester, Minn.

Oxidation of α-ketolic steroids with cupric acetate, on both micro and preparative scales, gives the corresponding glyoxals in high yield. This reaction has been adapted for the quantitation of α-ketols, since steroidal glyoxals react with the Porter-Silber reagent. In 100 µl of methanol were dissolved 4 to 60-µg amounts of corticosterone, 11-deoxycorticosterone, 11-epicorticosterone, and a number of other α-ketols saturated in ring A. To each was added 100 µl of 0.005M cupric acetate in methanol; after 30 to 60 minutes in air at room temperature, the solvent was evaporated. The Porter-Silber reaction was carried out on the residues in the usual way, except that heating was not required. Color development proceeded at room temperature and was maximal in 30 minutes. It was also possible to estimate 0.4 to 8.0-µg amounts of aldosterone similarly. All tests showed a linear relationship between the amount of α-ketol oxidized and color intensity over the above concentration ranges. As is true of the 17-hydroxy α-ketols, structural variations elsewhere in the molecule affected the chromogenicity of the α-ketol grouping.

2 *Pharmacology*

EFFECT OF C_{11}-SUBSTITUTION ON THE BIOLOGICAL ACTIVITY OF ADRENOCORTICAL STEROIDS. S. Tolksdorf*, F. Warren*, and P. L. Perlman. Schering Corporation, Bloomfield, N. J.

9α,11β-dihalocorticosteroids and 9α-halo-11β-acyloxycorticoids were tested for their selective effects on eosinopenic, anti-inflammatory and mineralocorticoid activity. The most potent compound in the acyloxy series was 9α-chloro-1,4-pregnadiene-11β,17α,21-triol-3,20-dione 11β-formate 21-acetate. Biological characteristics of this compound will be described. Further separation of anti-inflammatory from glucocorticoid as well as mineralocorticoid activity was observed in the 9α,11β-dihalocorticoid series. 9α,11β-dichloro-1,4-pregnadiene-17α,21-diol-3,20-dione was several times more active than prednisolone acetate in the granuloma pouch test and had very low eosinopenic and mineralocorticoid activities. In patients the dichloro steroid produced insignificant eosinopenic and anti-inflammatory effects by the oral route, but it showed excellent topical activity in allergic skin conditions. Data on other dihalocorticoids will also be presented.

3 *Pharmacology*

ANTI-INFLAMMATORY PROPERTIES OF COBALTOUS CHLORIDE AND 20-COBALTO-Δ-1-HYDROCORTISONE. James W. Fisher (intr. by Aaron Gans.) Dept. of Pharmacology, Univ. Tennessee Med. Units, Memphis 3, Tennessee.

The anti-inflammatory activities of hydrocortisone (I), cobaltous chloride hexahydrate (II), Δ-1-hydrocortisone (III) and 20-cobalto-Δ-1-hydrocortisone (IV) have been compared. Anti-inflammatory potency was measured using the granuloma pouch technique in intact female albino rats and the asbestos pellet granuloma test in adrenalectomized male albino rats. Subcutaneous injections of cobaltous chloride significantly ($P < .01$) reduced inflammation in intact rats. Infra-red spectrophotometry was used to study the chemical structure of the cobalt-Δ-1-hydrocortisone complex. The dosage of 20-cobalto-Δ-1-hydrocortisone necessary to reduce inflammation by 90% was significantly ($P < .01$) less and the slope of the dose-response curve was significantly ($P < .01$) greater than that of hydrocortisone, Δ-1-hydrocortisone, cobaltous chloride or simultaneous injections of cobaltous chloride and Δ-1-hydrocortisone at different s.c. sites. These data indicate that the addition of a cobalt atom at C20 of Δ-1-hydrocortisone significantly enhanced its anti-inflammatory potency. (Supported by USPHS Grant A-2973, Nat. Inst. of Arthritis and Metabolic Diseases).

4 *Pathology*

THE ACUTE EFFECT OF TYPHOID VACCINE ON THE RENAL INTERSTITIAL COMPARTMENT AND ITS PREVENTION BY CORTISONE. Irving Green (intr. by C.C. Erickson) Dept. Path., Med. Coll. Ga., Augusta.

C-14 labeled inulin was dissolved in radio-iodinated serum albumin and an aliquot injected rapidly into the renal artery. The renal venous washout was shunted via a cannula into tubes mounted on a moving kymograph. Aliquots of plasma were plated on planchettes and the radioactivity determined by passage through a flow-counter. Then the samples were covered by a planchette and again passed through the counter. I-131 counts were calculated from the shielded activity and the C-14 counts from the difference between the unshielded and shielded activity. Counts per min. were semilog. -plotted vs time from the mid-injection interval. Mean concentrations and mean transit times obtained from the I-131 washout curves were used to calculate plasma flow and volume. The same parameters obtained from C-14 washout curves were used to calculate the inulin (interstitial) "space". Experiments were performed on untreated dogs and on dogs given cortisone or typhoid vaccine or both. Washout curves were obtained in all cases 3 hrs. after the injections. The plasma washout pattern remained unchanged by the treatments described. However, the inulin washout curves following typhoid vaccine were greatly prolonged and contained multiple peaks. Cortisone, uneffective alone, prevented these alterations. (Supp. in part by a USPHS Grad. Train. Grant)

5 *Pathology*

THE EFFECT OF HYDROCORTISONE ON CULTURES OF HUMAN LIVER CELLS. John S. Wellington* and Henry D. Moon, Dept. of Pathology, Univ. of California School of Medicine, San Francisco, California

Human cells, derived from liver (Chang), were grown in Medium 199 containing hydrocortisone in concentrations ranging from 50 to 200mcg/ml. The cells of the control group were grown in Medium 199 alone. The cultures were incubated for 24 to 96 hrs. at 37°C. At the end of the experiment the number of cells and tyrosine content were determined in the experimental and control groups. In the group treated with hydrocortisone, the number of cells and tyrosine content were lower. These observations indicated that hydrocortisone inhibited nuclear multiplication and protein synthesis. With the higher concentrations of hydrocortisone the number of nuclei were proportionately fewer and the tyrosine values lower. The cells treated with hydrocortisone had more rounded outlines, were larger, exhibited greater anisonucleosis and had fewer mitoses; the cytoplasm of these cells was increased in amount and was less chromatophilic. Histochemical studies demonstrated a decrease in succinic dehydrogenase activity in the cells treated with hydrocortisone. Acid and alkaline phosphatase, esterase, glucose-6-phosphatase and DPND were similar in the experimental and control groups. (Supported by USPHS Grant CY-3341 and ACS Grant No. P-113).

102. ENDOCRINES–Glucocorticoids–Action

Physiology

ROLE OF THE INCUBATING MEDIUM IN EPINEPHRINE AND HYDROCORTISONE EFFECTS ON GLUCOSE UTILIZATION. M. Herman* and E. R. Raney. Dept. of Physiol., Georgetown Med. School, Washington, D.C.

The in vitro effect of epinephrine and hydrocortisone on glucose uptake by the isolated rat diaphragm was studied in the following media: (1.) Krebs-Henseleit bicarbonate, (2.) Krebs-Ringer phosphate containing .13 M NaCl, .005 M KCl, .003 M $CaCl_2$, .001 M $MgSO_4$, .01 M Na_2HPO_4 and .002 M HCl, (3.) Krebs-Ringer phosphate-low Ca, containing .001 M $CaCl_2$ and otherwise identical with (2.), (4.) Krebs-Ringer phosphate-high Mg, containing .002 M $MgSO_4$ and otherwise identical with (3.). All media were adjusted to a pH of 7.4 and contained 140 mgs of glucose per 100 ml initially. The control uptake of glucose was higher in the bicarbonate or the high Mg phosphate buffers than in the high or low Ca phosphate media. The addition of epinephrine to the medium (2 µg/ml) significantly inhibited glucose uptake by diaphragms incubated in media (2.) and (3.) but had no effect in media (1.) and (4.). Hydrocortisone (40 µg/ml) inhibited glucose uptake in the low Ca phosphate medium but was ineffective in the bicarbonate or high Mg phosphate media. The uptake of glucose and the inhibitory effect of epinephrine were indistinguishable in media (2.) and (3.). The dependence of phosphofructokinase activity on the concentration of Mg^{++} may in part account for these findings. (*Predoctoral Fellow, Natl. Inst. of Arthritis and Metabolic Diseases.)

2 **Physiology**

STUDIES ON THE IN VITRO REACTION BETWEEN LEUCOCYTES AND HYDROCORTISONE. Melvin M. Ketchel and Somers H. Sturgis (intr. by Ruth Conklin). Peter Bent Brigham Hospital and Harvard Medical School, Boston, Mass.

The amoeboid migration of leucocytes in clotted plasma is inhibited if 10 meg/ml of hydrocortisone (HC) is added to the plasma. We attempted to determine whether it is necessary for the HC to be present while the leucocytes are migrating, or if a previous exposure to HC will suffice. Leucocytes were incubated with HC for specific periods of time, washed to remove excess HC, and allowed to migrate in plasma which had no added HC. The inhibition was as large as that observed when HC was present while the leucocytes were migrating. Inhibition was observed in leucocytes incubated with HC for five minutes. Longer incubation did not increase the inhibition. Leucocytes washed virtually free of plasma proteins, and incubated with the HC in a protein-free salt solution were also inhibited when returned to plasma to migrate. Indicating that the hormone need not be bound to a specific plasma protein in order to be psysiologically active.

3 **Physiology**

A NEW FAMILY OF STEROIDS WITH STRONG ANTI-INFLAMMATORY ACTIVITY. J. G. Llaurado* and J. A. Schneider, Department of Macrobiology, Research Laboratories, Chas. Pfizer & Co., Inc., Groton, Connecticut

Introduction of a methyl group in C-21 of the molecule of 9α-fluoro-prednisolone (E. J. Agnello et. al., J.A.C.S., 1960, in press) has been shown to alter profoundly the biological characteristics of the parent compound. Compound P-1742 is 21-methyl-9α-fluoro-1,4,-pregndiene-11β,17α,21"β"-triol-3,20-dione 21-acetate and compound P-1822 is the 21"α" epimer of P-1742. The marked sodium-retaining activity of 9α-fluoro-prednisolone was abolished in the new compounds, while the anti-inflammatory properties were essentially preserved. Actual diuretic and natriuretic responses were observed with doses known to have strong anti-inflammatory effects as assessed by the granuloma pouch technique in the rat. The results clearly indicate that 21-methylation preserved or increased the strong anti-inflammatory action of the parent compound. A comparison of the biological spectrum of activity of 21-methyl derivatives with other anti-inflammatory steroids will be presented.

4 **Physiology**

CORTICOSTEROIDS AND INCORPORATION OF C^{14} INTO NUCLEIC ACID BY ISOLATED RAT DIAPHRAGM. Lloyd A. Ferguson and Ira G. Wool (intr. by J. O. Hutchens.) Dept. of Physiology, Univ. of Chicago, Chicago.

The effect of adrenalectomy and cortisone administration (2 mg/day) on nucleic acid synthesis was studied using the isolated rat diaphragm incubated with C^{14} adenine or thymine. When adenine-8-C^{14} (1 μc/ml; 8.33 x 10^{-5} M) was the substrate, incorporation of C^{14} into the nucleic acid fraction of diaphragm from normal rats was 5551 ± 234 (18)*; adrenalectomy enhanced (9673 ± 526 (16)) and cortisone depressed (4460 ± 189 (18)) incorporation. These changes parallel alterations in protein biosynthesis (Wool and Weinshelbaum, Am. J. Physiol. 197: 1089, 1959). Considerably less radioactivity was incorporated into muscle nucleic acid when incubation was with thymine-2-C^{14} (1 μc/ml; 10.47 x 10^{-5} M); control values were 195 ± 50 (6) and incorporation was not significantly influenced by adrenalectomy (162 ± 35 (6)) or by cortisone (147 ± 40 (6)). Apparently DNA turnover in skeletal muscle is less rapid than that of RNA. DNA biosynthesis is uninfluenced by corticosteroids.

*Results are expressed as cpm/100 mg diaphragm (wet weight). Values are mean ± S.E. of mean; number of observations in parentheses.

5 **Biochemistry**

The Effect of Steroid Hormones on the Rate of Reaction of L-Glutamic Acid Dehydrogenase. Lewis L. Engel, Jesse F. Scott* and Roberta F. Colman,* Huntington Mem. Labs., Mass. General Hosp., Depts. of Biol. Chem. and Anat., Harvard Med. School, Boston, Mass.

It has been shown that progesterone, deoxycorticosterone and testosterone form molecular complexes with adenine and compounds containing adenine. The present experiments were designed to explore the possible effects of such complex formation on enzymatic reactions. The initial rate of the reaction catalyzed by beef liver glutamic acid dehydrogenase, in which DPN was the coenzyme, was measured at 37° by following the change in absorbance at 340 mμ at 15-second intervals for the first 3 minutes of the reaction. It was found that when DPN was rate limiting, the conversion of glutamate to α-ketoglutarate was increased 10-15% by the presence of corticosterone (10^{-8}M), or by cortisol (10^{-6}M). The effects of these steroids were not additive. Testosterone (10^{-6}M) had no effect on the reaction rate, but when present together with corticosterone, it abolished the rate accelerating effect of the latter steroid. 11-Epicorticosterone, a biologically inactive isomer, was without effect in this system. When DPNH was rate limiting, the rate of the reverse reaction was decreased 8% by corticosterone and by cortisol. Deoxycorticosterone (10^{-6}M) was without effect.

6 **Pharmacology**

ACTICK OF ADRENAL STEROIDS ON HELA CELLS. Catherine C. Snell* and Allan D. Bass. Dept. of Pharmacology, Vanderbilt Univ. School of Medicine, Nashville, Tenn.

It has been shown in this laboratory that hydrocortisone sodium succinate (HC) and desoxycortisone acetate (DOCA) produce characteristic changes in HeLa cells grown in tissue culture. Specifically, these changes, produced by HC (50 μg/ml.) in eight day cultures include an increase in cell size, a decrease in number of cells per milliliter, an increase in total protein per flask, and an increase in protein, ribonucleic acid and desoxyribonucleic acid per cell. By treating the cultures with colchicine and HC for ten hours it was found that HC caused a 44% reduction in the mitotic index. DOCA(10 μg/ml. dissolved in propylene glycol) produced a similar increase in protein per cell and decrease in the mitotic index. Pilocarpine (0.2 μg/ml.) added to the cultures along with HC reversed the antimitotic action. Whereas pilocarpine alone produced no cellular effects. However, when pilocarpine was added to the substrate for eight days the above actions of HC were no longer modified.

7 **Pathology**

MEDIATION OF PHOSPHATURIA IN HYDROCORTISONE-INJECTED RATS BY PARATHORMONE. R. Arison* and H. C. Stoerk. Merck Institute for Therapeutic Research, Rahway, New Jersey.

It has been shown by Pitts that phosphaturia in hydrocortisone (Compound F) injected dogs is due to inhibition of tubular reabsorption of phosphate (TRP). This effect was long known to be typical of parathormone (PTH) activity. Therefore, urinary PO_4 excretion and TRP was studied in Compound F-injected, parathyroidectomized (PTX) rats. Confirming Pitts' findings in dogs it was observed that in Compound F-treated rats TRP is also inhibited. In PTX rats this effect of the steroid, over a dose range from 5-50 mg./kg., was not obtained. However, with extremely large quantities of Compound F (100 mg./kg.) phosphaturia due to inhibition of TRP occurred in absence of the parathyroids. Graded doses of parathyroid extract (PTE) caused progressive lowering of the percent TRP. Twenty mg./kg. of Compound F did not potentiate the effect of PTE. The injection of 100 mg. of Compound F caused a reduction of 20% in TRP which was additive to that of PTE when the steroid was given together with the extract.

XUM

8 *Pathology*

PANCREATIC ISLET CELL HYPERPLASIA IN HAMSTERS TREATED WITH CORTISONE AND CHLOROTHIAZIDE (DIURIL). J. K. Frenkel. Dept. Path. & Oncology, Univ. Kansas Sch. Med., Kansas City, Kansas.

Grossly enlarged and hyperemic islets were observed when chlorothiazide (D) was administered in addition to cortisone (Eac) and desoxycorticosterone (DOC) during a study of dissecting aortic aneurysms occurring in hamsters (Fed. Proc. 18, 477.#1879.). Eac 5 mg./wk. gave rise to 2X enlargement of islets and the addition of 1-5 mg./day D resulted in 3-10X beta cell hyperplasia, with ultimate hydropic change and cytolysis of islets, leading to replacement of up to half of acinar portion after 1-2 months. DOC or D, alone or together, were ineffective; DOC did not potentiate Eac.

PZ insulin 4 u/day delayed, and 8 u prevented hyperplasia. 2% NaCl or 2% KCl in drinking water or 1% Ca lactate in food were without effect, whereas .5% ZnO in food, or NaCl, KCl, Ca and Zn together hastened onset of islet hyperplasia resulting from Eac and D. When given with Eac only, NaCa versinate likewise gave rise to marked hyperplasia, whereas 1-5 mg. acetazolamide (Diamox), or 30-210 mg.% Na sulfadiazine in water were only slightly effective and 1-6 mg. tolbutamide (Orinase) did not potentiate Eac. Cortisol 7.5 mg. over 6 days, followed by 5 mg. D p.o., caused a rise in blood sugar reaching 600-800 mg.% in 6 hours in 70% of hamsters. (Supported by grant #E-1644 from U.S.P.H.S.)

103. ENDOCRINES–Glucocorticoids
Biosynthesis

1 *Physiology*

PRODUCTS OF CHOLESTEROL METABOLISM IN SOLUBLE EXTRACTS OF ACETONE-DRIED BOVINE ADRENOCORTICAL MITOCHONDRIA. I.D.K. Halkerston*, J. Eichhorn* and O. Hechter; Worcester Foundation for Experimental Biology, Shrewsbury, Mass.

In previous work we described a TPNH dependent soluble enzyme system derived from acetone-dried bovine adrenocortical mitochondria which converts C^{14}-cholesterol to progesterone. As we were unable to detect pregnenolone, we concluded that the soluble extract contained 3β-OH dehydrogenase as well as C-20,22 desmolase activity. Further study of various powder preparations has shown that both pregnenolone and progesterone in varying ratios may be obtained from C^{14}-cholesterol. We have now demonstrated that (a) the preparations convert pregnenolone to progesterone in the presence of DPN (3β-OH dehydrogenase activity); (b) the C^{14} products derived from C^{14}-cholesterol depend upon the available cofactors (endogenous and exogenous) present, so that under certain conditions cholesterol is converted almost exclusively to pregnenolone when endogenous DPN is not available whereas in other powders both pregnenolone and progesterone may be formed when endogenous DPN is available. Similar results are obtained with partially dialyzed acetone powders. (Supported by U.S.P.H. Service grant No. RG-4749).

2 *Physiology*

STIMULATION OF STEROID FORMATION OF ADRENOCORTICAL HOMOGENATES BY MEDULLA AND CATECHOLAMINES. D.Y. Cooper, V.J.Pileggi,* O. Foroff,* and Otto Rosenthal. Harrison Dept. Surg. Res., Sch. of Med., U. of Pa., Phila., Pa.

Studies of in vitro corticosteroid synthesis of adrenal slices indicated that slices containing medulla and cortex produced more steroids than cortex slices although medulla slices were inactive. We have now studied the action of H_2O homogenates of medulla upon synthesis of 17,21 di-hydroxycorticoids (Silber-Porter chromogens) by H_2O homogenates of cortex from steer adrenals in the following system: Krebs Ringer-bicarbonate-2% albumin (7 ml); progesterone (1 μmole); glucose-6-phosphate (24 μmole); TPN (2.4 μmole); cortical homogenates (appr. 100 mg); gas phase 5% CO_2 in O_2; 37°; 4 hrs. Addition of 15-30 mg of medulla increased steroid formation by 30-100%. Larger additions (up to 120 mg) produced additional smaller increments. Substitution of catecholamines (0.3 μmole of either epinephrine, norepinephrine or isoproterenol) for medulla also caused increments of from 30-100%. Larger amounts produced no additional increments. The studies show that catecholamines accelerate hydroxylation of progesterone, explaining in part the activating effect of medullary homogenates. (Supported by U.S.P.H.S. CY-3644 and S.E. Penna. Chapter of the Am. Heart Assoc.).

3 *Biochemistry*

STIMULATION OF CORTICOSTEROID FORMATION IN VITRO BY SERUM Rhoda Makoff* and Sidney Roberts. Dept. of Physiological Chem., Univ. of Calif., Los Angeles.

Previous studies have shown that specific proteins or protein-bound substances in rat serum stimulated release of corticosterone (B) and desoxycorticosterone (DOC) from surviving rat adrenal glands (Roberts, Ciba Found. Coll. on Endo. 11, 167, 1957). These studies did not differentiate between steroid release and biosynthesis, nor was the site of action of the serum factors determined. In the present studies, quartered rat adrenal glands were incubated with progesterone-4-C^{14}. The medium was then subjected to paper chromatography. Radioactivity was measured in the separated steroids directly on the paper strip. After elution the steroids were estimated colorimetrically. Added corticotropin (10 mu) stimulated release of B plus DOC but did not significantly increase their isotope content. Specific activity of B and DOC was decreased, substantiating Hechter's conclusion that ACTH stimulates corticosteroidogenesis at steps prior to progesterone conversion. Added rat serum (0.1 ml.) also increased release of B plus DOC. In contrast to the ACTH effect, conversion of radioactive progesterone to B and DOC was significantly stimulated. The results indicate that some factor in serum stimulates conversion of progesterone to corticosteroids.

4 *Pharmacology*

INFLUENCE OF VARIOUS INCUBATION MEDIA ON STEROID C-17 HYDROXYLATION. F.G. Hofmann. Coll. of Physicians & Surgeons, Columbia Univ., N.Y., N.Y.

Homogenates of guinea pig adrenal glands were prepared in four different media: hypotonic sucrose(0.025M) or KCl(0.015M) or isotonic sucrose(0.25M) or KCl(0.15M). The media were buffered with M/150 phosphate buffer(pH 7.2). Following centrifugation at 15,000 x g, the supernatants were supplemented with DPN,ATP, nicotinamide and progesterone -4-C^{14}(0.0001 M), and incubated for 2 hours at 37°C. Unlabeled desoxycorticosterone (DOC) and Reichstein's Substance S(S) were then added to the flasks and the conversion products extracted with CH_2Cl_2.These two compounds were isolated by paper chromatographic techniques and their specific activities determined. (In the absence of appropriate exogenous substrate, endogenous synthesis of these two steroids is negligible). Progesterone was converted to DOC to the same extent regardless of the incubation medium employed. Progesterone was converted to S to the same extent in all of the media except hypotonic KCl; C-17 hydroxylation appeared significantly depressed in this medium. Of note was the finding that there was no corresponding increase in the yield of DOC in this situation. Eichhorn & Hechter (Arch. Biochem. Biophys. 84:196,1959) likewise found C-17 hydroxylation depressed in a KCL solution but they found the inhibition to occur in the isotonic, rather than the hypotonic, medium.

104. ENDOCRINES–Glucocorticoids
Metabolism

1 *Physiology*

HORMONAL EFFECT ON STEROID CONJUGATION. D. L. Berliner & T. F. Dougherty, U. Utah, Salt Lake City, Utah.

Studies in hepatectomized animals have shown that the liver is the main organ responsible for reduction of ring A & conjugation of steroids. The capacity of liver to reduce ring A & conjugate steroids to water soluble compounds is altered by L & D triiodotyronine (T3) & ACTH. Both L & D-T3 increase the conjugation of corticosteroids having αβ unsaturated ketone structure in ring A but not of steroids reduced in ring A. ACTH & stress inhibit conjugation of both reduced and non-reduced ring A corticosteroids. The separation of the cells responsible for producing this effect has been attempted by utilizing the phagocytic properties of the Kupfer cells. By a modification of the technique of Rous & Beard animals were infused with iron & the iron-containing Kupfer cells were separated with a magnet. Both Kupfer & parenchymal cells were incubated separately in the presence of steroids having intact & reduced ring A. It has been shown that parenchymal cells mainly conjugate these steroids, but the Kupfer cell can oxidize & reduce the substituted groups of the steroid molecule. These transformations will be compared with those of fibroblasts. (Supported by Dept. of Army #DA-49-007-MD-130).

2 **Biochemistry**

ROLE OF TRIPHOSPHOPYRIDINENUCLEOTIDE IN THE METABOLISM OF 4-C^{14}-CORTISOL BY MUSCLE TISSUE. Max L. Sweat, Dept. Obstetrics & Gynecol., U. Utah, S. L.C., Utah 4-C^{14}-cortisol-(F_k)-incubated with mouse muscle mince at 37°C for 1-1/2 hrs in 0.05 M phosphate buffer (pH 7.4) was metabolized to cortisone (E_k), Reichstein's substance E (E_r), dihydro-F_k, corticosterone (B_k), & 11-β-hydroxyandrostenedione. Secondary products via E_k were Reichstein's substance U(U_r), 4-5-dihydro E_k, 11-dehydro B_k-(A_k)-and adrenosterone. (Methodology: Anal. Chem. 28:1608'56, 29:1797'57 & Biochem. Biophys. Acta. 28:591'59). TPN but not DPN significantly increased the quantity of E_k, E_r & dihydro F_k. A soluble preparation of muscle 11-cortisoldehydrogenase studied at pH 6.2, 7.4, & 9.2 demonstrated TPN specificity. (No evidence for a transhydrogenase). The major product of E_k in muscle is U_r; in liver it is F_k. The conservation of C-11-OH as noted by Bush & Mahesh (Biochem. J. 71:718'59)-appears to be primarily a hepatic function not a peripheral one. If the mechanism of action of cortisol is via a chemical reaction, it is possible that one(or more) of these reactions observed in muscle is associated with the key hormonal process(es) of cortisol (Supported by U.S.P.H.S. grants Rf-4720, A-1803).

3 **Biochemistry**

DISTRIBUTION AND METABOLISM OF CORTICOSTEROIDS IN LIVER AND MUSCLE CELL FRACTIONS OF THE RAT. F. DeVenuto,* P. C. Kelleher* and U. Westphal. US Army Med. Rsch. Lab., Fort Knox, Ky.

The distribution of cortisol-4-C^{14} in liver and muscle cell fractions of the normal and adrenalectomized rat was studied in vivo and in vitro. The liver cell components were separated and washed by "equilibrium fractionation" using 0.44 M sucrose solution. Of the original radioactivity of the liver, 15-23% was found in the mitochondria, 5-8% in the nuclei, 2-3% in the microsomes, the supernatant containing the remaining portion. With corticosterone-4-C^{14}, similar results were obtained. No appreciable amount of radioactivity was found in the rat muscle cell fractions when the distribution of cortisol-4-C^{14} was studied; the main portion (85%) of the radioactivity remained in the supernatant. Whereas mitochondria and microsomes of the liver cell transformed cortisol-4-C^{14} to compounds more polar and less polar than cortisol, no metabolism of cortisol occurred in the cytochondrial fraction of the muscle cell. The separation and identification of the various metabolites will be discussed.

4 **Biochemistry**

PREPARATION OF DERIVATIVES OF STEROID HORMONES BY DIRECT CHROMIC ACID OXIDATION ON PAPER. Frank M. Genis (intr. by F. L. Haven.) Depts. of Radiation Biology and Biochemistry, Univ. of Rochester School of Medicine and Dentistry, Rochester, New York.

The technique to be described involves a direct treatment of paper chromatographic fractions, containing isolated steroid hormones, with a solution of boiling CrO_3 in 90% acetic acid. A special apparatus is used which permits not only the oxidation, but also subsequent elution of the derivative. After oxidation and elution, the eluate is chromatographed directly on paper in appropriate solvent systems for complete characterization of the oxidized product. The technique has been successfully used for the identification of steroid hormone metabolites obtained by perfusion of kidney and incubation of adrenal tissue. (This paper is based on work performed under contract with the U. S. Atomic Energy Commission at the University of Rochester Atomic Energy Project.)

5 **Pharmacology**

BLOOD LEVELS, TISSUE DEPOSITION, EXCRETION, AND METABOLISM OF A SYNTHETIC CORTICOSTEROID (TRIAMCINOLONE). J. R. Florini, E. A. Peets, and D. A. Buyske (intr. by Earl H. Dearborn), Experimental Therapeutics Research, Lederle Laboratories Division, American Cyanamid Company, Pearl River, New York.

The plasma half-life of tritium labeled triamcinolone (9α-fluoro-11β,16α,17α,21-tetrahydroxy-1,4-pregnadiene-3,20-dione) was 116 ± 7.4 (S.E.M.) minutes following intravenous injection at a dose of 2 mg./kg. in six dogs. Red blood cells contained half as much ethyl acetate-extractable tritium as the pooled plasma and cell washings. The labeled steroid was deposited very rapidly in the tissues of the rat following intravenous administration. Approximately 60% of the injected radioactivity was found in muscle 5 minutes after dose. Maximum liver concentration (15% of dose) occurred at 30 minutes. Radioactivity was detectable in tissues and blood 48 hours after injection. At least 85-90% of injected tritium was excreted by the dog with the radioactivity being equally divided between urine and feces. Approximately 20% of the injected steroid was excreted unchanged in the urine within 8 hours of dosing at 2 mg./kg. The urinary metabolites differ markedly from those observed with other members of the hydrocortisone family; no reduction of the A ring or side chain was detected and no glucuronide conjugation could be demonstrated.

6 **Pharmacology**

ENDOCRINE AND SPECIES INFLUENCES ON CORTISONE METABOLISM IN VITRO. A. A. Hagen* and R. C. Troop. Dept. of Pharmacology, Univ. of Tenn. Med. Units, Memphis.

Whole liver homogenates from male and female rats are known to metabolize the 17,21-dihydroxy-20-ketone sidechain and Δ4-3 ketone group of cortisone in a manner characteristic of each sex. Sidechain reduction is more rapid in the male; A-ring reduction more rapid in the female. The effects of castration, age, and gonadal hormone administration on the hepatic systems responsible for cortisone reduction have also been studied. This report discusses other endocrine influences on these systems. Cortisone acetate (5 mg/d/10d/IM) reduced C-20-keto reductase activity in both males and females but Δ4-3 ketone reduction was depressed in males only. Neither pregnancy nor progesterone administration significantly influenced cortisone metabolism in homogenates from female rats. Liver homogenates from 12 male and female dogs did not show a sex difference in reduction of the side chain or A-ring. The rates of cortisone reduction expressed as ug/200mg. liver /2 hrs. are: Sidechain-male 62 ±3.7, female 65 ±6.1; A-ring-male 25 ± 5.1, female 20 ± 5.2. This suggests that sidechain reduction may be more important than A-ring reduction in the inactivation of cortisone by the dog.
Supported by USPHS Grant A-2750.

106. ENDOCRINES–Insulin

1 **Physiology**

INSULIN SENSITIZATION TO K-STROPHANTIN ACTION. V.W. Adamkiewicz (intr. by E. Pagé), Laboratoire de Physiologie, Université de Montréal, Canada.

Insulin-Zn (40 u/kg. s.c.) prolongs 2-4 folds the cardiac action of the glycoside k-strophantin (10-20 mg/kg. s.c. or i.p.) in the rat (100-200 g.), as reflected by the duration and intensity of the heart rate deceleration, and as measured with an E.C.G. Insulin also increases by 20% the mortality of rats slightly overdosed with k-strophantin (30 mg/kg. i.p.) but simultaneously retards sixfolds the time of death. On the other hand, insulin appears not to affect the cardiac action of the aglucon strophantidin. The glycoside k-strophantin is a derivative of glucose, as is also the glucan Dextran. The influence of insulin on the biological action of the glucan was reported previously. The similarity between the influence of insulin on the biological action of the glucoside and of the glucan is now illustrated. (Supported by grant No. A-2260 from the U.S. Department of Health, Education and Welfare and grant No. MA 640 from the National Research Council of Canada.)

XUM

2 *Physiology*

INSULIN EFFECTS ON THE TURNOVER AND OXIDATION OF GLUCOSE IN THE HUMAN AS STUDIED WITH C^{14} GLUCOSE. G. L. Searle, L. R. Bristow* and W. A. Reilly*. Radioisotope and Medical Services, Vetrans Administration Hospital, San Francisco 21, California.

In a study comparing the effects of insulin and tolbutamide on the oxidation of glucose in the human it was noted that although insulin caused a greater transient increase in the removal of glucose from the plasma, the increase noted in the amount of glucose oxidized to CO_2 was equal with both substances. This evidence suggested to us the possibility of demonstrating an action of insulin wherin a significant increase in the oxidation of glucose may occur with only a minor increase evidenced in the turnover rate of glucose. Diabetic and non-diabetic subjects are being studied before and after graded doses of insulin. Each subject is studied for a period of at least three and one half hours before the administration of insulin to establish base line values for the rates of turnover and oxidation of glucose, and for at least three hours after the administration of insulin to determine the effects of insulin on the rates of turnover and oxidation of glucose. Preliminary results from these studies have shown that it is possible to demonstrate significant increases in the rate of glucose oxidation while at the same time the turnover rate of glucose is affected to a minor degree following the administration of insulin.

3 *Biochemistry*

ELECTROPHORESIS OF THE REACTION PRODUCTS OF INSULIN IN UREA SOLUTION. Fritz Bischoff and Abolfath Khan Bakhtiar*. Santa Barbara Cottage Hosp. Research Inst., Santa Barbara, Calif.

Regular crystalline insulin and the five reaction products isolated when insulin reacts in concentrated urea solution (J. Am. Chem. Soc. 78:1343, 1956) were subjected to electrophoresis in the P. E.- Tiselius apparatus at pH 8.6 (barbital buffer), ionic strength 0.1, 1°C. Insulin OU, which forms on precipitating insulin immediately from the urea solution by water, and insulin IU, which has lost its ability to crystallize but not its hypoglycemic response, developed major and minor peaks with corresponding mobilities in the electrophoretic pattern identical with those observed for intact crystalline insulin (J. Am. Chem. Soc. 75:3121, 1953) and confirmed by us. Insulin IIU, which has a delayed biologic response even on intravenous injection, insulin IIIU which has half the biologic activity of IIU, and insulin IVU which is devoid of biologic activity had major peaks with respective mobilities ranging from -7.8 to -9.1 X 10^{-5} cm/sec/v/cm. After precipitating IIU as the sulfate, the electrophoretic pattern changed (even after prolonged dialysis), the major and minor peaks respectively having mobilities of -6.6 and -7.6 X 10^{-5}. Mixtures of serum albumin and crystalline insulin were not resolved on electrophoresis. (Aided by the Carbon P. and Bertha E. Dubbs Foundation. Insulin, gift of Eli Lilly and Company.)

4 *Biochemistry*

INSULIN BIO-ASSAY BY GLYCOGEN DEPOSITION IN A SINGLE RAT DIAPHRAGM. D. C. Jessup and G. S. Wiberg (intr. by L. I. Pugsley.) Dept. of Natl. Health and Welfare, Ottawa, Canada.

Various assay procedures have been described which measure insulin activity using glucose uptake by the isolated diaphragm as the criterion of response. Criticisms of these methods include the variation occurring between animals and the non-specificity of glucose uptake as a measure of insulin activity. Pooling tissues partially overcomes the between animals variation but it introduces another source of error, namely the inability to detect non-viable tissue which may be present. A procedure has been developed which uses portions of a single rat diaphragm and employs glycogen synthesis as a specific response to insulin. Unlike glucose uptake, glycogen formation in the isolated diaphragm does not appear to be affected by the presence of cut tissue. The use of a single rat diaphragm eliminates the between animals variation and provides a simple, economical technique. The procedure consists of cutting the diaphragm into 8, approximately equal, parts and incubating each section in glucose saline. Individual glycogen concentrations are determined for each tissue section. A linear relationship exists between the log. of the insulin concentration and the amount of glycogen deposited. As little as 10-25 micro-units of insulin per ml. cause a significant increase in glycogen deposition.

5 *Nutrition*

THE HUMAN REQUIREMENT FOR VITAMIN B6. Irvin C. Plough (introduced by H. E. Sauberlich). U. S. Army Medical Research and Nutrition Laboratory, Fitzsimons Army Hospital, Denver, Colorado.

A group of young men have been fed for 2-3 weeks, a series of diets, both synthetic and otherwise, in which the vitamin B6 content measured by microbiological assay varied from 0 to 7 mg. per day. With intakes of 2.0 mg. of B6 per day or less, there were definite increases in urinary excretion of xanthurenic acid following ingestion of 10 gm. dl-tryptophane, as compared with intakes greater than 2.0 mg. of B6. Under the same conditions, there were no changes in the levels of serum transaminases, nor were any clinical changes observed that could be related to the dietary B6 intakes. These findings justify setting the "maximal" minimum daily requirement of humans for vitamin B6 at about 2.5 mg. It seems likely however, that the requirement to maintain health is much lower, for the tryptophane load test probably puts heavier stress on a single biochemical pathway than can ever normally occur.

6 *Nutrition*

INFLUENCE OF ANABOLIC STEROIDS ON THE PROTEIN CATABOLIC ACTION OF GLUCAGON. Stanley R. Glasser and Joseph L. Izzo (intro. by W. S. McCann). Depts. of Radiation Biol. and Medicine, Univ. of Rochester School of Med. and Dent., Rochester, N.Y.

Possible suppression of the protein catabolic action of glucagon by androgens has been investigated in female rats fasted 3 days. Control rats were compared with rats receiving 0.75 mg crystalline glucagon b.i.d., with rats receiving either 0.075 mg norethandrolone, b.i.d. or 0.075 mg testosterone propionate, b.i.d. and with rats receiving glucagon in combination with either androgen. Total urinary nitrogen was determined daily. After 3 days fast animals were sacrificed and blood α-amino N, urea N and glucose as well as liver and muscle glycogen were analyzed. Glucagon-treated rats showed the characteristic rise in urinary N excretion and fall in blood α-amino N compared to controls, but the hepatic glycogenolytic action of glucagon noted after 5 days fast (Fed. Proc., 17:78 #306, 1958)was not apparent after 3 days.Neither the synthetic androgen, norethandrolone, nor the natural androgen, testosterone, caused any nitrogen retention in the fasting rat. Furthermore, when given in combination with glucagon both hormones failed to suppress the protein catabolic action of glucagon.(Supported by grants from U.S.P.H.S.A-3556, USAEC #W-7401-eng-49 and Eli Lilly Co.).

107. ENDOCRINES–Insulin–Action

1 *Physiology*

ACTION OF INSULIN ON ADIPOSE TISSUE FROM HYPERTHYROID RATS. Jean Himms Hagen (intr. by J. Doupe). Dept. of Physiology, University of Manitoba Medical School, Winnipeg, Manitoba.

Effects of insulin on glucose uptake, CO_2 production (as measured by overall gas exchange), lactic acid output and NEFA release by epididymal adipose tissue from normal and hyperthyroid rats have been measured. Adipose tissue, from rats weighing between 150 and 250 g, was incubated in Krebs' bicarbonate Ringer solution containing 5% albumin and 0.01M glucose with or without insulin, 0.1U per ml. Unstimulated glucose uptake was the same in normal and hyperthyroid rats. Stimulation by insulin of glucose uptake and CO_2 output was greater in hyperthyroid rats than in normal rats whether expressed in terms of tissue wet weight or of tissue nitrogen. After insulin the CO_2 output was greatly in excess of that equivalent to O_2 consumption. In both the normal and hyperthyroid tissues the excess CO_2 was related mole for mole to the extra glucose uptake. Insulin had little or no effect on lactic acid production and NEFA release. It is concluded that the ability of insulin to stimulate glucose uptake, glucose oxidation and, presumably, fatty acid synthesis is greater in hyperthyroid rats than in normal rats.

2 **Physiology**

GLUCOSE UPTAKE BY CHICK EMBRYO HEARTS IN VITRO. G. Guidotti*, D. Kanazeishi* and P. P. Foà. Dept. of Physiology and Pharmacology, Chicago Medical School, Chicago 12, Ill.

Glucose uptake by chick embryo hearts at various stages of development was studied in vitro with or without insulin. Glucose uptake is greater in anaerobiosis than in aerobiosis and decreases in both as hearts develop. Insulin has no effect on uptake by 5-day old hearts but causes a 20 to 40% increase in 8 to 10-day old hearts. The appearance of insulin sensitivity coincides with the embryologic development of the pancreatic islets. Mincing the hearts does not modify uptake by 5-day old hearts, increases that of 9-day old hearts, but abolishes insulin effect. Thus, insulin responsiveness may depend upon the development of an insulin-sensitive "membrane". Uptake increases with glucose concentration in the medium: in 5-day old, insulin-insensitive hearts a maximum is reached with 0.004 - 0.008 M concentrations; in 9-day old, insulin-sensitive hearts no maximum is reached with concentrations as high as 0.032 M. In 3-hr experiments the rate of uptake with or without insulin decreases slightly as incubation proceeds and glucose concentration in the medium decreases. The effects of insulin concentration and of insulin antisera are being studied. Aided by grants from the Chicago Heart Association and the USPHS (A-522).

3 **Physiology**

FURTHER STUDIES ON THE EFFECT OF INSULIN ON GLUCOSE PRODUCTION. A. Dunn,* R. Steele,* N. Altszuler, D. T. Armstrong,* J. S. Bishop,* and R. C. de Bodo. Dept. of Pharmacology, New York Univ. Coll. of Med., New York City, and Biology Dept., Brookhaven Natl. Lab., Upton, N. Y.

Earlier studies in the dog using a C^{14} glucose priming injection-continuous infusion technique, revealed a small transient decrease in glucose production immediately following insulin administration and a "restraining" influence during insulin infusion on the increase in glucose production in response to hypoglycemia (Ann. N. Y. Acad. Sci. 82:431, 1959). These studies were extended by investigating the effect of insulin on the markedly elevated glucose production induced by phloridzin. Phloridzin (1 gm in 1% Na_2CO_3, sc.) injection in normal dog 2 hrs. prior to the C^{14} glucose experiment led to marked glucosuria, however a near normal plasma glucose concentration was maintained by a several fold increase in hepatic glucose output. Intravenous infusion of insulin reduced this elevated glucose production to about the normal rate. Upon termination of the insulin infusion, the glucose production returned to its high pre-insulin rate. Whether or not this influence of insulin in reducing hepatic glucose output is exerted directly on the liver, and whether glycogenolysis or gluconeogenesis is affected is being investigated.

4 **Physiology**

INSULIN EFFECT ON MUSCLE POTASSIUM FLUX. Kenneth L. Zierler. Johns Hopkins Univ. School of Med., Baltimore, Md.

The net effect of insulin on potassium is a decrease in extracellular K and an increase in intramuscular K concentration. To see whether this was due to increased K influx or decreased K efflux, K fluxes were measured with K^{42} in excised rat extensor digitorum longus muscle. In balanced salt solutions, whether glucose was present or absent, insulin decreased both K influx and efflux but the decrease in efflux was greater, yielding net intramuscular accumulation of K. When half the Cl was removed from the bathing solution, K efflux and influx decreased equally and there was no change in membrane potential. The data are compatible with the hypothesis that insulin increases the positive fixed charge on the membrane. (Performed under contract with ONR and supported by grant from Muscular Dystrophy Assns.)

5 **Physiology**

GLUCOSE UPTAKE BY THE ISOLATED, PERFUSED HEART FROM HYPOPHYSECTOMIZED RATS; THE EFFECTS OF INSULIN AND GROWTH HORMONE. M. J. Henderson, H. E. Morgan, and C. R. Park, Physiology, Vanderbilt, Nashville.

The uptake of glucose by the isolated, perfused heart from hypophysectomized (hypex) rats has been measured over a range of perfusate glucose concentrations. Uptake is reduced to about 70 per cent of the rate in the normal heart. This is due to a reduced capacity (V_{max}) of membrane glucose transport which is the major limiting step for the uptake process. When insulin is added transport is greatly accelerated and phosphorylation becomes principally limiting. Phosphorylation, determined as a function of intracellular glucose concentration, is not changed from normal and is not grossly affected by insulin in vitro. Transport is more sensitive than normal to low levels of insulin and is stimulated by the insulin-like activity of growth hormone in vitro whereas normal tissue is refractory. Following growth hormone in vivo, the isolated heart of the hypex rat shows accelerated transport and uptake at one hour but 24-48 hours later the control rate is reestablished.

6 **Physiology**

EFFECT OF INSULIN ON HEPATIC CARBOHYDRATE AND FAT METABOLISM IN NORMAL AND DEPANCREATIZED DOGS. W.C.Shoemaker* J.Ashmore, P.J.Carruthers* I.C.Powers* M.Schulman* and H.Feinberg*. (Intrd. by H. Necheles) Michael Reese Hosp. and Med. Center. Chicago, Illinois

The rate of hepatic uptake or output of plasma glucose, non-esterified fatty acids, total fatty acids, cholesterol, cholesterol esters and phospholipids, were measured in a series of unanesthetized, normal and depancreatized dogs whose hepatic vessels were previously catheterized. Hepatic blod flow was measured by a modified and improved bromsulphalein method. HGF-free insulin (Lilly) was administered by constant infusion into the portal vein at the rate of .0016 to .0033 u/kg/min. The mean arterial glucose concentration of 11 experiments on normal dogs fell from 88 mgm/100ml in the control period to an average of 75 mgm/100ml during the first hour of insulin infusion. The mean control hepatic glucose output was 80± 3.8 (S.E.) mgm/min; after insulin this averaged 112± 21 mgm/min. There was also no essential change in the diabetic series. The hepatic uptake of non-esterified fatty acids increased from .05 meq/min in the control period to .150 meq/min after insulin (P=.001). An increase in hepatic uptake of free cholesterol and an increase in output of total fatty acid was also observed after insulin.

7 **Physiology**

INSULIN-LIKE ACTIVITY OF NORMAL HUMAN SERA. Beigelman, Paul M. (intr. by S.Sobin.) U. So. Cal. Med. Sch., Los Angeles, Cal.

This report is concerned with bio-assay studies of insulin-like activity in normal human serum. The particular bio-assay technique employed utilizes glucose uptake by rat epididymal adipose tissue. This method is highly sensitive, detecting as little as 10 micro-units of insulin/ml., but does not possess a high degree of precision. Pooled human serum demonstrated decline of insulin-like activity proportional to the extent of dilution, marked activity being present in undiluted serum and serum diluted 1/4. Only doubtful activity was evident at 1/10 dilution and none at 1/100. 47 presumably normal individuals were tested for serum insulin-like activity The time interval after eating was found to be important, with highest activity noted about 1-2 hours post-prandial. Oral glucose tolerance tests, probably more precise, indicated maximum activity 1/2-1 hour post-prandial. Considerable individual variation was observed, attributable in part to variability of the method, but also suggesting marked inherent individual variations.

XUM

8 *Physiology*

HUMORAL CHARACTER OF THE HYPOGLYCEMIC EFFECT OF MUSCULAR WORK. M. S. Goldstein. Michael Reese Hospital, Chicago, Illinois.

Strenuously contracting muscles lead to increased glucose uptake even in the absence of insulin. This has been shown to be the result of increased entry of glucose into the cells and not a direct result of increased metabolic transformation of the carbohydrate. This increased rate of cellular penetration of sugars is shared by a variety of non-utilizable carbohydrates in a manner parallel to that demonstrated for insulin. Using cross-transfusion techniques and using the transfer of thoracic duct lymph from working to resting animal preparations, it has been shown that strenuously contracting muscles release a humoral material which effects this increased rate of sugar penetration of cells. Chronically depancreatized dogs are capable of elaborating this insulin-like hypoglycemic factor of striated muscle.

9 *Biochemistry*

EFFECTS OF ALLOXAN DIABETES ON THE TWO GLYCOLYTIC PATHWAYS IN RAT DIAPHRAGM. Walter N. Shaw, Albert I. Winegrad and F.D.W. Lukens (intr. by Carl Bachman.) John Herr Musser Dept. Res. Med. and Cox Res. Inst., Univ. of Pennsylvania, Phila.

The coexistence of two Embden-Meyerhof pathways in normal rat diaphragm which differ in their response to insulin in vitro has been described (J. Biol. Chem. 227:115, 1957; 234:2491, 1959). These two pathways were identified as the result of differences in response to insulin of normal diaphragms incubated in phosphate buffer and in bicarbonate buffer. These pathways have been studied in the diaphragms of alloxan diabetic rats. In these diaphragms lactate production from glucose in the phosphate or bicarbonate buffer is normal. Insulin in vitro has no effect on lactate production in either medium while glycogen synthesis is increased. Fructose-DP isolated from washed diaphragm is not formed from medium glucose in either buffer. When diaphragms from alloxan diabetic rats are incubated with C^{14} glucose in either buffer and unlabeled fructose-DP is added, total lactate production is increased but the incorporation of glucose carbon into lactate is decreased. Thus, in disphragms from alloxan diabetic rats, lactate is formed by a pathway which has no barrier to the entrance of glucose and is not insulin responsive.

10 *Biochemistry*

GLUCOSE PRODUCTION AND PLASMA NON-ESTERIFIED FATTY ACID LEVELS FOLLOWING INSULIN ADMINISTRATION. D.T.Armstrong*, R.Steele, N.Altszuler†, A.Dunn*, J.S.Bishop*, and R.C.DeBodo.* Biology Dept., Brookhaven Nat. Lab., Upton, N.Y. and Pharmacology Dept, New York Univ. Coll. of Med., New York, N. Y.

Hypoglycemia, induced in normal dogs by continuous infusion of insulin (.07-.17 units/kg/hr i.v.), was shown by an isotope dilution technique to result primarily from increased glucose utilization, and was accompanied by an initial decline in plasma non-esterified fatty acid (NEFA) level. During mild hypoglycemia, plasma NEFA remained low, while hepatic glucose output continued at normal or slightly increased rates as long as the insulin infusion continued. Upon termination of the insulin infusion, NEFA returned toward normal with a concomitant increase in rate of glucose production. With more severe hypoglycemia, a greater increase in glucose production appeared during insulin infusion, accompanied by increased plasma NEFA. Termination of insulin infusion then resulted in an additional increase in glucose production although no further NEFA elevation occurred. Insulin is thought to lower plasma NEFA by increasing the glucose available to adipose tissue cells. The present experiments show that plasma NEFA may be restored to normal while glucose utilization is still accelerated, and that this occurs simultaneously with an increased glucose production.

11 *Biochemistry*

A NON-TRANSPORT EFFECT OF INSULIN ON PROTEIN SYNTHESIS. M. Toyoda* and S.P. Bessman, Univ. of Md. Sch. of Med., Balto., Maryland.

Previous work showed that insulin stimulation of glycogen synthesis in rat diaphragm is manifest under conditions precluding an effect on transport of carbohydrate (Fed. Proc. 17, 190, 1958, 18, 192, 1959). Further evidence for independence of the anabolic action of insulin from transport has been obtained by studying amino acid incorporation into free and protein pools in rat muscle. Diaphragm, whole, halves, and pieces, was incubated with C^{14} valine or tryptophan and free and protein bound radioactivity were measured. Insulin had the usual stimulatory effect on protein incorporation of the amino acids. The appearance of radioactivity in the protein free supernatant was independent of insulin, however, and the insulin treated tissue showed a slightly smaller amount of non-protein radioactivity than the controls. The data do not support the transport hypothesis that insulin stimulates protein synthesis by the production of a high intracellular amino acid content which exerts a mass action effect. The data are compatible with an intracellular role of insulin and with the above mentioned studies on glycogen synthesis.

12 *Biochemistry*

INSULIN TREATMENT AND UDPG GLYCOGEN TRANSGLUCOSYLASE ACTIVITY. C. Villar-Palasi* and J. Larner. Western Reserve Univ. School of Med., Cleveland, O.

Previous findings led to the postulation of an insulin effect on glycogen synthesis (Ann.N.Y.Acad. Sci. 82, 345 (1959)). To examine directly the question of an insulin-mediated enzymatic effect phosphoglucomutase, UDPG pyrophosphorylase, UDPG glycogen transglucosylase, and phosphorylase activities were compared in extracts prepared from hemidiaphragms incubated with and without added insulin. We report here that UDPG glycogen transglucosylase activity is increased in extracts prepared from hemidiaphragms incubated with insulin over controls. No differences in activities of the other three enzymes of the glycogen cycle were noted. Retention of increased activity after ammonium sulfate precipitation and wash rules out a loosely bound co-factor. Since UDPG glycogen transglucosylase in skeletal muscle appears to be rate-limiting in the synthetic reaction sequence of the glycogen cycle as measured in broken cell preparations, it is conceivable that increased activity of this enzyme could lead to increased synthesis of glycogen and increased glycogen content.

13 *Biochemistry*

THE MECHANISM OF TOLBUTAMIDE INDUCED HYPOGLYCEMIA IN THE DOG. J. R. Leonards, J. W. Craig*, F. J. R. Martin*, F. Barry*, and M. Miller*. Depts. of Biochem, Medicine and Surgery, Western Reserve U., Cleveland, O.

Hepatic glucose output (HGO) was determined in the triple cannulated dog (Shoemaker et al., Am. J. Physiol. 196, 311 (1959)) both by hepatic-portal glucose gradients and by C^{14} glucose kinetics. Following the intravenous injection of 30 mg/kg of tolbutamide in 10 dogs there was a diminished HGO in only one instance. In 7 cases there was an increased rate of isotope disappearance from the blood interpreted as an accelerated rate of peripheral utilization of glucose. Increased disappearance in the other experiments may have been masked by release of isotope incorporated into liver glycogen prior to the tolbutamide. When non-labeled glucose was infused at a rate at least equivalent to the HGO of the dogs, tolbutamide still produced hypoglycemia as well as an increased rate of isotope disappearance. These observations are felt to be consonant with increased peripheral utilization of glucose as the predominant mechanism of tolbutamide action in the dog.

14 *Biochemistry*

Stimulation of the Stereospecific Sugar Transport System of Muscle by Uncouplers and SH-Inhibitors. Tetsuro Kono* and Sidney P. Colowick. School of Medicine, Vanderbilt University, Nashville, Tenn.

It is known that 2,4-dinitrophenol (DNP) and other agents promote the penetration of sugars into rat diaphragm muscle (Randle &Smith: Biochem. J., 1958). The present studies show that these effects are not due to destruction of the cell membrane, since the process retains the same stereospecificity as for insulin-stimulated sugar transport. Thus, DNP and p-hydroxymercuribenzoate (PHMB) promote penetration of D(+)-galactose, L(+)-arabinose and D(+)-xylose, at rates about one-half as high as with insulin, but have no detectable effect with D(-)-arabinose and L(-)-xylose. The same conclusion is reached by Morgan et al. (unpublished data cited by Park et al.: Am. J. Med., May, 1959). The effects of DNP and PHMB are not insulin dependent, since they are observed with diabetic muscle. These agents, although exerting an insulin-like effect, inhibited the effect of added insulin. The optimal concentration for the insulin-like effect of DNP is 10^{-4}M., whereas the concentration required for complete inhibition of added insulin is 10^{-3}M. Ultraviolet irradiation of diaphragm at 2570 Å gave effects qualitatively similar to, but less pronounced than those found with DNP.

108. ENDOCRINES–Insulin–Diabetes Mellitus

1 *Physiology*

SITE OF ORIGIN OF HYPERGLYCEMIC SUBSTANCES FOUND IN PANCREATO-DUODENAL BLOOD. J. W. Weber, J-P. Colombo, D. Kanameishi and P. P. Foà (intr. by J. A. Smith). Dept. of Physiology and Pharmacology, Chicago Medical School, Chicago 12, Ill.

At least 2 types of hyperglycemic materials appear in the pancreato-duodenal vein following i.v. injections of growth hormone (STH) or injections of deserpidine into the pancreato-duodenal artery. No hyperglycemia occurs when the pancreas is bypassed by injections into a mesenteric artery or the portal vein. Materials released by STH resemble serotonin or epinephrine because their action is blocked by dihydroergotamine (DHE); materials released by deserpidine are unaffected by DHE and resemble glucagon. To study if these substances derive from pancreas or duodenum, dogs were depancreatized leaving pancreato-duodenal artery and vein intact. Completeness of pancreatic removal was judged by development of ketosis and at autopsy. After recovery from surgery and ketosis and reduction of glycosuria to a minimum with insulin, deserpidine was injected into the duodenal artery under anesthesia. In contrast to normal dogs, no hyperglycemia was obtained. The results suggest that the hyperglycemic substance released by deserpidine derives from the pancreas. Aided by Grants A-522 and 2A-5102, USPHS.

2 *Physiology*

MANNOSE AND GLUCOSE METABOLISM BY ADIPOSE AND LIVER TISSUES OF NORMAL AND DIABETIC RATS. F. C. Wood, Jr.*, G. F. Cahill, Jr.* and A. E. Renold, Harvard Medical School, Boston, Mass.

Epididymal adipose tissue and liver slices from normal and alloxan diabetic rats were incubated with glucose and mannose randomly labeled with C^{14}. In normal adipose tissue the pattern of incorporation of substrate carbon into glycogen, glycerol (α carbons), fatty acids and CO_2 was essentially the same for the two hexoses. The ratio of recovered mannose carbon to that of glucose was .82. Insulin stimulated the metabolism of both hexoses, again resulting in similar metabolic patterns. The insulin effect on mannose (10 mM) was not significantly altered by the addition of an equimolar concentration of unlabeled glucose. The metabolism of mannose by liver slices was again similar to that of glucose, and again somewhat slower. The incorporation of mannose carbon into fatty acids, CO_2, glycerol and glycogen by adipose tissue from diabetic rats was 0, 6, 15 and 40 per cent of normal, with corresponding values for glucose of 0, 6, 11 and 47 per cent of normal. Liver slices from diabetic rats metabolized mannose carbon to CO_2, fatty acids and glycogen 44, 16 and 6 per cent as well as normal tissues, the comparable glucose figures being 25, 18 and 4 per cent respectively. Mannose and glucose thus appear to be metabolized by very similar pathways, similarly affected by insulin.

3 *Physiology*

EXTENT OF CONVERSION OF C^{14}-LACTATE AND C^{14}-PYRUVATE TO GLUCOSE IN HUMANS. R. C. DeMeutter and W. W. Shreeve. (intr. by I. L. Schwartz.) Brookhaven National Laboratory, Upton, N. Y.

An injection of 40 to 100 μcs. of 2- or 3-C^{14}-DL-lactate or 2- or 3-C^{14}-pyruvate was given i.v. to normal subjects and diabetics of different types. Blood samples were taken at intervals over a two-hour period. C^{14} in blood glucose was determined by combustion of its osazone to CO_2. A rapid and extensive conversion of C^{14} to glucose was observed in diabetics without treatment; 1 hour after injection the amount of C^{14} in free body glucose ranged from 15 to 30% of the dose given. After i.v. injection of insulin or (in the stable diabetic) tolbutamide 10 to 20 min. prior to another dose of the same tracer, the specific activity as well as the total amount of C^{14} found in glucose was consistently and substantially decreased. It is concluded that in diabetic humans gluconeogenesis via lactate and pyruvate is an active metabolic process. During the period of action of insulin or tolbutamide no complete halt of production or outflow of glucose occurs, but the findings suggest some early effect on the production or release of glucose by the liver. (Supported by the Atomic Energy Commission.)

4 *Biochemistry*

METABOLIC STUDIES OF TISSUES FROM PREGNANT, ALLOXAN-DIABETIC RATS. Dwain D. Hagerman and Frederica M. Wellington*. Dept. of Biological Chemistry, Harvard Medical School, Boston, Mass.

Earlier tissue slice incubation experiments in a buffered phospho-saline medium (Fed.Proc. 17:236, 1958) were extended by incubating slices of maternal and fetal liver and placenta from normal and alloxan-diabetic rats in a high potassium, bicarbonate medium, pH 7.4, with glucose-U-C^{14} and pyruvate-2-C^{14} as substrates. Replacement of Na^+ by K^+ in the medium led to decreased rates of glycogenolysis and glucose production and increased rates of glucose oxidation to carbon dioxide in maternal liver. Pregnancy alone increased the rate of glycolysis and lipid synthesis from pyruvate by maternal liver, while the rate of conversion of pyruvate to glucose was decreased. In the pregnant rat diabetes decreased glucose utilization and oxidation to carbon dioxide, and lipid synthesis from glucose by both maternal and fetal liver. Placentas from the diabetic animals contained and utilized much more glycogen, utilized less glucose and converted less glucose to carbon dioxide and lipid than the controls. The results are compatible with the view that the changes in placental metabolism are the result of an absolute insulin deficiency while the fetal liver shows changes, perhaps adaptive, due to a relative insulin deficiency in the face of an overwhelming supply of glucose from the mother.

5 *Nutrition*

IN VITRO EFFECT OF CHROMIUM(III) ON GLUCOSE UPTAKE OF FAT TISSUE. W. Mertz and K. Schwarz. Natl. Insts. of Health, Bethesda, Md.

The glucose tolerance factor (GTF), a dietary agent required for maintenance of normal glucose tolerance in the rat, has been identified with trivalent chromium (K. Schwarz and W. Mertz, Arch. Biochem. Biophys. 85, 292, 1959). When suitable chromium(III) complexes are used, the impairment of glucose removal is cured by a single dose of 20 μg of the element. The development of low glucose removal rates is prevented by chromium(III) supplements to GTF-deficient diets. The in vitro effect of chromium(III) on the uptake of glucose by epididymal fat tissue was investigated by the technique of Winegrad and Renold. Ca. 100 mg of tissue was incubated in 2 cc of fresh, oxygenated Krebs-Ringer phosphate buffer containing 100 mg% glucose. In the absence of insulin, no significant effect of chromium(III), added as chrome alum, was seen. In the presence of 1 milliunit of crystalline Zn-insulin, .1 μg of chromium(III) enhanced glucose removal by 73%. In 10 experiments, average glucose uptake per 100 mg tissue and hr. amounted to 41 ± 6 μg glucose without, and 71 ± 9 μg with chromium supplement. Similar results were seen with C^{14}-labelled glucose. Lower - and also higher - doses of chromium(III) gave lesser effects. It is concluded that in this system the effect of chromium is closely associated with the action of insulin, and that it affects the utilization of glucose by fat tissue.

109. ENDOCRINES–Insulin–Secretion

1 *Physiology*

CHANGES IN OUTPUT PER MINUTE OF PANCREATIC VENOUS INSULIN-LIKE ACTIVITY FOLLOWING HYPERGLYCEMIA IN THE DOG. R.H.Egdahl and H.L.Goldberg*. Surg. Res. Lab., Med. Coll. Va., Richmond, Va.

The present experiments were designed to quantitatively measure the output per minute of insulin-like activity from an isolated pancreatic segment in the dog. A plastic cannula was introduced into the pancreatoduodenal vein and a choker device was placed proximally. Duodenal branches of the vein were ligated. Intermittent samples of uncontaminated pancreatic venous blood were obtained by choker occlusion. Insulin-like activity was determined by the epididymal fat pad method of Martin and Renold (1958). Insulin-like activity is consistently lower in peripheral than in pancreatic venous blood. Significant increases occur in both peripheral and pancreatic venous blood insulin-like activity following induced hyperglycemia. The output per minute of insulin-like activity from the pancreas increased severalfold. Studies on hepatic venous output per minute of insulin-like activity suggest that the liver may help to regulate glucose metabolism by "trapping" and releasing insulin.

2 *Biochemistry*

INCREASE IN SERUM INSULIN-LIKE ACTIVITY IN RATS WITH GROWTH. J. Oyama* and R. L. Grant. Div. of Pharmacology, Food & Drug Admin., U. S. Dept. of Health, Education and Welfare, Washington, D. C.

From measurements of serum insulin-like activity of various species by the mouse diaphragm technique (Proc. Soc. Exp. Biol. Med. 100: 90, 1959) the mean activity was found to increase in the order: human <mouse, rabbit, dog <<rat. The high activity of rat serum led us to determine this insulin-like activity in rats (Osborne-Mendel) from weaning to maturity. Blood samples were obtained by cardiac puncture from 24 hour fasted rats anesthetized with sodium pentobarbital. The mean serum activity (increased glucose uptake in mg./10 mg. dry wt. of diaphragm tissue/90 min.) showed a marked rise from a minimum value at 3 weeks of 0.011±0.005 to 0.030±0.010 at 5 weeks and 0.083±0.010 at 7½ weeks. The last value approximates the activity found in mature rats. Comparison of fasted and non-fasted rats (3 week old) showed the latter to have a significantly higher mean activity of 0.040±0.009.

3 *Biochemistry*

AN IMMUNOCHEMICAL ASSAY OF HUMAN INSULIN. G.M. Grodsky, P.H. Forsham, and C.T. Peng (intr. by H. Harper). Metabolic Unit, Univ. of Calif. School of Medicine, San Francisco, Calif.

Given amounts of insulin-I^{131} and antisera (guinea pig) are incubated, and the bound insulin is determined after preferential precipitation with $NaSO_3$. Percent binding decreases with increasing unlabeled insulin added as standards or unknowns. By pre-extraction of serum with acid alcohol, 80% of insulin was routinely obtained irrespective of any endogenous insulin antibody. The method is, therefore, applicable to subjects given previous insulin therapy. Values obtained by the immunological and a mouse convulsion assay (Eli Lilly & Co.) of insulin extracted from normal human pancreas were 61.5 μg./ml. ± 15.2% and 58.7 μg./ml. ± 8.4% respectively, indicating the near quantitative cross reaction between extracted human insulin and antibodies produced against beef insulin. By either method, a human islet cell tumor contained 10 times as much insulin. Normal insulin circulating levels in human and beef serum were lower than the minimum sensitivity of the method (20 μ-units/ml.). Elevated insulin levels (30-50 μ-units/ml.) occurred in most subjects 30 min. after oral glucose. A resistant diabetic given 500 units insulin retained 3-6 milliunits/ml. 24 hours after injection despite concurrent hyperglycemia, glycosuria and ketonuria. This method appears applicable to the chemical measurement of total circulating insulin in man.

110. ENDOCRINES–Mineral Corticoids

1 *Physiology*

RELATION OF ANTERIOR PITUITARY FUNCTION TO ALDOSTERONE AND CORTICOSTERONE SECRETION IN CONSCIOUS DOGS. James O. Davis, Charles C.J. Carpenter*, Carlos R. Ayers* and Robert C. Bahn, Natl. Heart Institute, Bethesda, Md. and Mayo Clinic, Rochester, Minn.

Steroids were measured in adrenal vein plasma obtained with a chronic indwelling catheter. In conscious normal dogs, secretion of aldosterone and corticosterone was lower in many instances than in normal anesthetized dogs following the "stress" of acute adrenal vein cannulation. In conscious dogs with thoracic caval constriction, aldosterone secretion was markedly increased above normal, whereas corticosterone output was frequently very low. However, in dogs not well trained or excitable, secretion of steroids was elevated above the basal output. Hypophysectomy of dogs with thoracic caval constriction resulted in a greater, more abrupt fall in corticosterone output than in aldosterone secretion. In 2 of these hypophysectomized dogs, aldosterone secretion failed to return to normal for several days; during this period, hyperaldosteronuria and Na retention continued. In another animal, aldosterone secretion fell markedly within 2 days after hypophysectomy and a natriuresis ensued. The data show that hyperaldosteronism occurred 1) in the presence of low as well as high secretion rates of corticosterone and 2) after hypophysectomy before aldosterone output declined to normal or below. The anterior pituitary provided important support for a high rate of aldosterone production.

2 *Biochemistry*

ALDOSTERONE EXCRETION IN HYPERTENSION. Josephine B. Garst, N. P. Shumway*, H. Schwartz*, and G. L. Farrell*. Crile Veterans Administration Hospital and Western Reserve School of Medicine, Cleveland, Ohio.

Twenty-four hour urinary aldosterone excretion was determined on 38 patients with hypertension and 18 normotensive controls. No patient was included who showed evidence of frank renal, liver, or endocrine disease, or cardiac failure. The 24-hr. urinary aldosterone values of the controls appeared to be normally distributed with a mean of 9.07 μg./24 hrs., S.D., 2.81. The 24-hr. urinary aldosterone values for the hypertensives considered as a whole averaged 13.29 μg./24 hrs., S.D., 9.11. However, the values for the hypertensives appeared to fall into two groups. The majority (75%) were distributed in the same way as the normotensive values, but a second, smaller group showed definitely elevated aldosterone excretion levels (above 17.5 μg./24 hrs.). Chi square calculation and t test confirmed the suspected heterogeneity of the hypertensive group. Study of the effect of an aldosterone antagonist is under way in those patients with elevated aldosterone output and normal serum potassium.

3 *Biochemistry*

EFFECT OF AN ALDOSTERONE ANTAGONIST ON GASTRIC AND RENAL FUNCTION IN DOG. Helena Gilder, Charles McSherry and John M. Beal, (intr. by V. duVigneaud) Cornell Univ. Med. Coll., New York, N. Y.

Previous experimental work with Aldosterone antagonists, such as SC-8109 (3-(3-oxo-17β-hydroxy-19-nor-4-androsten-17α-yl) propionic acid γ-lactone) have tended to show that they antagonize the effects of aldosterone on the renal distal tubule to produce increased Na excretion. Nembutalized dogs with Heidenheim pouches were used to compare the effect of SC-8109 on gastric juice and urine. The clearance of water, electrolytes, and inulin, and the distribution of injected Evans blue, Na^{24}, and $S^{35}O_4$, were determined before and after the intravenous injection of the drug. An immediate rise of gastric juice Na, Cl, and volume occurred, with little effect on K or free acid. The effect lasted about 2 hours. In contrast, urine K increased and urine Na and volume were unchanged in the first 2 hours, after which marked diuresis and saluresis occurred. Others have shown that SC-8109 stimulates the adrenal to produce increased aldosterone. This may explain the initial sodium retention by the kidney. The experiments suggest the possibility that aldosterone acts on tissues other than the kidney. (Supported by P.H.S. grant no. RG 4942.)

4 *Pharmacology*

FATE AND METABOLISM OF ALDOSTERONE-H^3 IN ADRENALECTOMIZED RATS. Herbert Sheppard, Alfred A. Renzi and Nathalie R. Bowen (intr. by Albert J. Plummer.) Research Dept., CIBA Pharmaceutical Products Inc., Summit, N. J.

Aldosterone randomly labeled with tritium was administered subcutaneously at a dose of 10 μg/rat to 4 rats which had been adrenalectomized 24 hours previously. Two of the 4 were also treated with a spirolactone (SC-8109) at a dose of 13 mg/rat. One hour later the animals were given a 0.2% saline load. Urine was collected during the pre- and post-loading periods. Tritium analyses were done on several fractions of urine and tissues and some general results were as follows: 1) No effect of the spirolactone could be detected on the distribution and metabolism of the tritiated aldosterone; 2) Over the total 3-hour collection period a total of approximately 13% of injected dose (I.D.) was excreted in the urine; 3) The G.I. tract contained approximately 45% of the I.D. with over 75% of this radioactivity present in the caecum; 4) Approximately 8% of the I.D. was found in the liver with less than 1% in the spleen, heart, brain, muscle or kidney; 5) Over 50% of the radioactivity in the G.I. tract is derived from some material which is water soluble and not hydrolyzable with acid or β-glucuronidase.

111. ENDOCRINES–Posterior Pituitary

1 *Physiology*

PHARMACOLOGICAL CHARACTERIZATION AND TENTATIVE IDENTIFICATION OF NEUROHYPOPHYSIAL HORMONES IN FISH. Wilbur H. Sawyer, Robert A. Munsick* and H. B. van Dyke. Dept. Pharmacology, Columbia Univ. College of Physicians and Surgeons, New York.

The fowl neurohypophysis contains two active peptides. One appears to be oxytocin. The second has pharmacological characteristics identical to those of arginine vasotocin, a peptide synthesized by Katsoyannis and du Vigneaud. We have applied rat vasopressor, i.v. rat antidiuretic, rat uterus in vitro, rabbit milk-ejection, hen oviduct in vitro and isolated bullfrog bladder assays to extracts from a marine teleost (Pollachius virens), an elasmobranch (Squalus acanthias) and a cyclostome (Petromyzon marinus). Activities in Pollachius extract are consistent with the assumption that it contains both arginine vasotocin and oxytocin. Petromyzon extract appears to contain arginine vasotocin but little or no oxytocin. Squalus extracts follow a pattern of activities that cannot be readily explained by assuming the presence of any neurohypophysial peptide or analogue that we have yet studied. Tentative conclusions can be drawn concerning the evolutionary distribution of neurohypophysial peptides. (Supported in part by Grants No. A1940 and H1788 from the U.S.P.H.S. and a grant from the N.Y. Heart Association).

2 *Physiology*

METABOLISM OF ANTIDIURETIC HORMONE IN DOGS. H. D. Lauson and M. Bocanegra.* Albert Einstein College of Medicine, New York.

In dogs, external jugular blood after severe hemorrhage is rich in antidiuretic activity. The fate of this natural antidiuretic hormone (ADH) was studied by bioassay in conscious, hydrated female dogs. Antidiuresis was evaluated from changes in urine flow and osmolality, and free water, osmolal, and endogenous creatinine clearances. ADH activity of blood was found only in plasma, none in red cells. ADH concentration in ultrafiltrates of plasma was 70-80 per cent of that in impermeates. Infusion of ADH-rich plasma into the left renal artery (method of Rudolph et al., Proc. Soc. Exp. Biol. and Med., 93, 323, 1956) induced less antidiuresis in the right kidney than infusion of the same dose into a foreleg vein; renal clearance of ADH was equivalent to about 20-30 per cent of renal plasma flow. In analogous studies, hepatic portal clearance of ADH was equivalent to about 5-15 per cent of hepatic portal plasma flow. Total plasma clearance of exogenous bovine Pitressin (plasma ADH concentration at high levels) in pentobarbital-anesthetized dogs was equivalent to about 40-65 per cent of renal plasma flow. If total plasma clearance of endogenous ADH is similar to that of exogenous Pitressin, most, or perhaps all, of ADH removal could be attributed to the kidneys and liver. (Supported by grants RG-4986 and A-1588 from the Public Health Service.)

3 *Physiology*

RENAL ACTIVITY OF VASOPRESSIN IN ANESTHETIZED DOGS. Joseph H. Perlmutt. Univ. of N. C. School of Medicine, Chapel Hill.

Water diuresis was produced in anesthetized dogs by infusing glucose in water at 5 ml/min. After suitable control periods, various doses of vasopressin were infused for 2½ hrs. to determine an effective dose. 50 mU/hr. consistently produced decreased urine flow, a concomitant sustained increase in Na excretion and negative free-water clearance. HCO_3 excretion usually exceeded Cl excretion and urinary pH rose. Tubular rejection of HCO_3, however, is unessential for vasopressin action since antidiuresis was obtained in an acidotic dog. Glomerular filtration rate (GFR) increased from 3-23% above control values. In another group of dogs the hydrating fluid was administered at 10 ml/min. For significant antidiuresis, 100 mU/hr. were usually required. Na excretion rose during the first hour of vasopressin infusion and was accompanied almost entirely by Cl, while during the next 1½ hrs. Na and Cl excretion declined despite some elevation of GFR. These data show that vasopressin administered to anesthetized dogs in water diuresis produced its usual antidiuretic effect and that different electrolyte excretory patterns occurred depending on the degree of hydration. Whether or not these results were due primarily to vasopressin or secondarily to altered body fluid volume and composition remains to be determined. (Supported by National Heart Institute Grant H-2457)

4 *Pharmacology*

RELEASE OF POSTERIOR PITUITARY HORMONE IN RATS BY NICOTINE AND LOBELINE. R. S. Grewal,* F. C. Lu and M. G. Allmark.* Food & Drug Laboratories, Dept. of National Health & Welfare, Ottawa.

Nicotine and hence tobacco smoking have been shown to cause a release of posterior pituitary hormone (Brit. J. Pharmacol. 6: 471, 1951) which has a strong coronary constrictor action. Since lobeline, a nicotine-like substance, has been recommended as an agent to curb smoking, some experiments were carried out to determine its activity, if any, in releasing this hormone. Two groups of 4 rats each after heparinization were given respectively, lobeline and nicotine, I.P., at 1 mg/100 gm. The rats were killed by quick decapitation and the blood collected from the rats of each group was pooled. The posterior pituitary hormone was extracted from the refrigerated plasma using a modification of the method of Walker and Risset (J. Physiol. 126: 588, 1954). The amount of this hormone in the extract was assayed against a reference standard using the rat blood pressure method (Brit. J. Pharmacol. 7: 567, 1952) and the 1 X 2 dose design (J. Am. Pharm. A. 32: 138, 1943). With this procedure the recovery of added hormone was 90-100%. In a series of experiments, lobeline was found to release 2-3 mU of this hormone per ml of blood, as compared to 3-4 mU with nicotine. In an isolated perfused rat heart, an injection of 4 mU of this hormone reduced the coronary flow by 25%. The antidiuretic activity of lobeline was about 1/8 that of nicotine in the rat.

112. ENDOCRINES–Sex Hormones

1 *Physiology*

CHARACTERIZATION OF MAMMALIAN SEX HORMONES IN THE INVERTEBRATES. Charles R. Botticelli, Frederick L. Hisaw, Jr., and Herbert H. Wotiz, (intr. by F. L. Hisaw.) Biol. Labs., Harvard Univ., Cambridge, Mass., Dept. of Biochemistry, Boston Univ. School of Medicine, and Univ. of Washington Marine Labs., Friday Harbor, Washington.

The ovaries of the starfish, Pisaster ochraceous, the sea urchin, Strongylocentrotus franciscanus, and the pecten, Pecten hericius, were extracted for steroids. Estrogenic activity equivalent to less than 10 μg. per kilogram of wet tissue was found in each species by bioassay. The estrogenic substance was identified as estradiol-17β by countercurrent distribution (29 transfers: upper phase 70% methanol-lower phase 50% chloroformic carbon tetrachloride), paper chromatography (3 systems: toluene-propylene glycol; ligroin-propylene glycol; benzene-formamide), and bioassay (Astwood method). Progesterone was also identified tentatively by column chromatography and the Hooker-Forbes assay. Other progestational substances were present, one of which may be Δ^4-3-keto-pregnen-20β-ol. The occurrence of these steroids in two distinctly different phyla indicates a wide distribution of these substances and may be of some significance in the evolution of sex hormones.

XUM

2 *Physiology*

WATER AND ELECTROLYTES IN RAT DECIDUOMA. Helene C. Cecil*, T. R. Wrenn*, Joel Bitman*, and J. F. Sykes. Dairy Cattle Research Branch, U. S. Dept. of Agriculture, Beltsville, Md.

Studies were made of H_2O, Na, K, and Cl in rat uteri with and without deciduoma and in uteri from ovariectomized rats. Samples of whole uterus, endometrium, and myometrium were obtained. The proportion of endometrium increased from 6.6% to 27.2% when deciduoma were present. Uterine horn weight increased from about 300 mg to over 900 mg. With the development of deciduoma the following effects occurred in whole uterus and myometrium:

1. H_2O content increased about 4%.
2. Na and Cl increased 22% on a dry weight basis, although there were no differences when based on wet weight.
3. K increased 16% on a wet and 40% on a dry weight basis.

The water and electrolyte content of uteri from ovariectomized rats was very similar to that of uteri without deciduoma. Endometrium could only be obtained from uteri with decidual tissue. H_2O, Na, and Cl were about equal to the concentrations in whole uterus with deciduoma, but K was considerably higher than that observed in whole uterus. The ionic changes which the rat uterus exhibits during the development of deciduoma are characteristic of tissue growth. Similar changes have been observed in uterine growth under the influence of progesterone in the rabbit and the cow.

3 *Biochemistry*

METABOLISM OF 19-NORTESTOSTERONE IN VITRO. D. Kupfer and E. Forchielli (intro. by M. Hayano). The Worcester Found. for Exper. Biol., Shrewsbury, Mass.

An in vivo study of 19-nortestosterone metabolism revealed a pattern similar to testosterone (Engel et al. J. Biol. Chem. 231, 159 (1958). This report concerns ~~with~~ the in vitro transformation of 19-nortestosterone by female rat liver. 19-Nortestosterone was incubated with a homogenate prepared in 0.05M phosphate buffer at pH 7.2 in the presence of TPN and air. The transformation products were extracted and separated with the Girard reagent into ketonic and non-ketonic fractions and isolated by paper and adsorption chromatography. The metabolites were identified by comparison of mixed melting points, infrared spectra and optical rotations. The ketonic components were identified as 3α-hydroxy-19-nor-5α-androstan-17-one (I) (m.p. 149, 166-7°C: $[\alpha]_D$ +110 in $CHCl_3$) and 3β-hydroxy-19-nor-5α-androstan-17-one (II) (m.p. 117-9°C: $[\alpha]_D$ + 108 in $CHCl_3$) and the non-ketonic components as 19-nor-5α-androstan-3α,17β-diol (III) (m.p. 192-193.5°C; $[\alpha]_D$ + 23.4 in $CHCl_3$ and 19-nor-5α-androstan-3β,17β-diol (IV). Cpd. (I) has been previously reported in in vivo studies; compounds II, III and IV are metabolites of 19-nortestosterone heretofore unreported. The syntheses of the new steroids I, II and III will be described briefly.

4 *Pharmacology*

AN EFFECT OF RESERPINE ON GROWTH AND SEXUAL MATURATION. Paul Hagen and A. C. Wallace.* Departments of Biochemistry and Pathology, University of Manitoba, Winnipeg, Canada.

Weekly injections of reserpine (4 mg./kg.) begun at one month of age inhibited growth and sexual maturation of male chickens. At 14 weeks of age, animals which had received reserpine weekly were about half the size of normal chickens (mean weight 704 gm. (S.D. ± 220 gm.) compared with 1407 gm. (S.D. ± 246 gm.) for controls). Reserpine also inhibited the development of the combs. Thus the mean comb weight of treated animals was 0.42 ± 0.3 gm. compared with 11.9 ± 5.5 gm. in controls. The mean weight of the testes of treated animals was 0.43 ± 0.67 gm. compared with 10.9 ± 6.7 gm. in controls. Histologically the testes of treated animals showed marked to extreme hypoplasia. The effect of 1 mg./kg. and 2 mg./kg. doses of reserpine weekly was only slightly less marked than the effect of 4 mg./kg. (Supported by a grant from the National Research Council of Canada and grant No. B 2282 from the U.S.P.H.S.)

5 *Pharmacology*

STUDIES ON THE FUNCTIONAL ULTRASTRUCTURE OF THE RAT VENTRAL PROSTATE. David Brandes, D.P. Groth, and Adolfo Portela (intr. by S.E. Mayer) Emory Univ. School of Med., Atlanta, Ga.

The fine structure of epithelial cells of the rat ventral prostate has been investigated with the electron microscope. In the normal male rat the endoplasmic reticulum consists of a very elaborate system of cavities, which show complex communications and a high degree of dilation. It was assumed that the material which filled these intracellular cavities constituted secretory products of these cells. Castration and estrogen administration caused a rapid collapse of these dilated cavities and a reduction in the small granules attached to their limiting membranes. Correlative studies on the rate of incorporation of glycine-2-C^{14} into the proteins of the ventral prostate will also be reported for the normal, castrate, and estrogen treated rat.

113. ENDOCRINES—Sex Hormones—Androgens

1 *Physiology*

PURIFICATION AND FURTHER CHARACTERIZATION OF THE TPN-SPECIFIC 17-β-HYDROXY (TESTOSTERONE) DEHYDROGENASE. Gerald L. Endahl* and Charles D. Kochakian. Univ. of Alabama Med. Center, Birmingham, Alabama.

Guinea pig liver has been previously reported to contain a TPN specific 17-β-hydroxy (testosterone) dehydrogenase which can be separated from a DPN-specific dehydrogenase by centrifugation at 24,500 xg and remains in the soluble fraction even after centrifugation at 105,400 xg for 3 hours. This enzyme now has been purified by ammonium sulfate (45-55%) precipitation, paper electrophoresis, and chromatography on a column of diethylaminocellulose. Electrophoresis indicated that the enzyme has a positive charge. The enzyme dehydrogenated only the 17-β-hydroxyl of C-19 steroids. It showed no activity toward the 3α and β hydroxy groups of several steroids. Michaelis-Menten constants were: Testosterone 5.6×10^{-5}; etiocholan-17-β-ol,3-one, 2.07×10^{-5} and androstan-17-β-ol,3-one, 1.08×10^{-5}. The purified preparation did not contain the TPN specific enzymes: glucose-6-phosphate dehydrogenase, isocitric dehydrogenase, and malic enzyme. Transhydrogenase activity also was absent. Mn^{++}, Ca^{++}, Al^{++}, Mg^{++} and Zn^{++} at 1×10^{-6} to 1×10^{-3} M had no influence on the activity of the enzyme. (Supported by grant CY-3566, National Cancer Inst.)

2 *Physiology*

ANDROGEN AND PROTEIN NUTRITIONAL INFLUENCES ON NITROGEN BALANCE. Paul F. Brande* and J. H. Leathem. Rutgers Univ., New Brunswick, N. J.

Adult male rats were fed an 18% protein diet for 20 days. Calorie intake was restricted to 100 calories/kg. Nitrogen balance/kg was 900 mg., 1800 mg., 3200 mg., and 4000 mg. for diets containing lactalbumin, wheat gluten, casein and gelatin respectively. Testosterone propionate (0.5 mg. daily) improved nitrogen balance in rats fed lactalbumin or wheat gluten only. The effect of nutritional variation on nitrogen balance was not reflected in protein concentrations of kidney, liver, heart and gastrocnemius. Protein concentrations of the heart and muscle were significantly less in hormone-treated rats fed lactalbumin than in androgen-treated rats fed the other proteins. The data provide further evidence that protein nutrition can influence hormone action.

Physiology

3

THE C^{14}-LABELED STEROID APPEARING IN RAT VENTRAL PROSTATE AFTER ADMINISTRATION OF TESTOSTERONE-4-C^{14}. B. W. Harding and L. T. Samuels. (intr. by Don H. Nelson.) Dept. of Biochemistry Univ. of Utah and the A.R.C. Unit of Reproductive Physiology and Biochemistry Univ. of Cambridge, England.

Although it is known that testosterone regulates the metabolism of male accessory reproductive tissue the nature of the hormone at its site of action has not been resolved. We have undertaken to identify the androgens and to determine their subcellular distribution in rat ventral prostate after I.V. infusion and I.P. injection of testosterone-4-C^{14}. The concentration of C^{14}-labeled steroid in the ventral prostate and blood was found to be the same. While testosterone was the major androgen identifiable in the blood and muscle, the only radioactive steroid found in the ventral prostate was Δ4-androstene-3,17-dione. This androgen was located largely in the microsomal and supernatant fractions. It is concluded that the interaction of testosterone with rat ventral prostate results in its oxidation to Δ4-androstene-3,17-dione and that this substance occurs primarily in the light particulate and soluble cell fractions where it is in rapid equilibrium with the blood testosterone. (Supported by a grant from the National Cancer Institute U.S.P.H.) We wish to thank Dr. T.R.R. Mann for his advice and continued interest.

Biochemistry

4

THE ANALYSIS OF HUMAN URINE FOR 3,5-CYCLOANDROSTAN-6β-OL-17-ONE. W. R. Nes, L. Segal, and B. Segal (intr. by R. I. Dorfman). Clark Univ., Worcester, Mass., and Worcester Foundation for Experimental Biology, Shrewsbury, Mass.

While certain procedures for the isolation of 17-ketosteroids from human urine are known to produce 3,5-cycloandrostan-6β-ol-17-one as an artifact of dehydroepiandrosterone (DHEA) sulfate, it is possible that other methods, involving aqueous acidic solutions, produce free DHEA as an artifact of the i-steroid. In order to clarify this problem, we have studied isolation procedures and found that the following method will cause neither the i-steroid nor the retro-i-steroid rearrangement. Urine containing added i-steroid and DHEA sulfate was neutralized, made 3 M with NaCl and extracted with ethyl acetate. The organic layer was dried with Na_2SO_4, made 2 x 10^{-4} M with H_2SO_4, and allowed to remain at 30° for 20 hrs. It was then extracted with 1 N NaOH, H_2O, dried, and the solvent was removed. The residue was chromatographed, and the i-steroid and DHEA were identified. The solvolysis is acid-catalyzed and inhibited by water. The half-time of reaction under the conditions described is 2 hrs., but it can be modified by adjustment of the H_2SO_4, H_2O, and temperature making the method applicable to varying laboratory requirements. (Supported by Grants #5001 and #6019 of the National Institutes of Health.)

Biochemistry

5

RELATION OF FRUCTOSE SYNTHESIS IN THE MALE SECONDARY SEX ORGANS TO ALDOSE AND KETOSE REDUCTASE ACTIVITIES. L.T.Samuels, B.W. Harding* and T.R. Mann.* Cambridge University, Cambridge, England, and University of Utah, Salt Lake City, Utah.

The concentration of fructose varies widely among different male secondary sex glands in the same species, and in the same gland among species. Since Hers showed that only aldose reductase, which converts glucose to sorbitol, and ketose reductase, which converts sorbitol to fructose, were involved in fructose synthesis by the sheep seminal vesicle, the activities of the two enzymes were studied in the various secondary sex glands of the rat and in the seminal vesicles (s.v.) of the sheep, bull, boar and horse. While in the rat the s.v., which does not produce fructose, has 3 times the ketose reductase activity of the coagulating gland, rich in fructose, it lacks significant aldose reductase. The ventral prostate has little of either enzyme. The sheep and bull s.v. have high activity of both enzymes. The boar s.v. has good aldose reductase activity but little ketose reductase. The horse s.v. is low in both. Thus the level of fructose production could be correlated with the activity of the least of the two enzymes. There was no direct correlation between the activities of the two enzymes and the degree of androgenic effect in calves of different ages. No evidence of androgen-sensitive transhydrogenase could be found.

Biochemistry

6

METABOLISM OF TESTOSTERONE-4-C^{14} IN A STEER. Robert P. Martin and F. X. Gassner (intr. by F. A. Cajori.) Endocrine Section, Colorado State University, Fort Collins, Colorado

A cross-bred steer was injected s.c. with 385 uC. (115 mg.) testosterone-4-C^{14}. Radioactivity in urine and feces and in biopsy samples of blood, muscle, and liver was determined over 119 days. The tissue levels were never higher than 6 ug. C_{19} steroid/100 g; after Day 7 they were below 0.6 ug. Of the dose, 8.4% was excreted via urine and 90% via feces. Half-life was 10 days, 3/4-life 26 days. Examination of the metabolites in Day 1 feces has been made. Benzene extracts of aqueous filtrates resulting from $Pb(OH)_2$ treatment of alcoholic extracts of the dried feces gave an over-all recovery of 90%. Most of this radioactivity as purified by column and paper chromatography appeared in 2 distinct zones on paper. The compounds in the less "polar" zone have not yet been identified. Essentially all of the radioactivity of the other zone has been identified as epitestosterone-4-C^{14} by mobility of the free compound and its acetate, UV absorption, Zimmermann reaction, crystallization with epitestosterone, and conversion by CrO_3 to a product which was identified as androstene-3,17-dione by chromatography and mixture crystallization. This suggests that in the bull a means of deactivation of endogenous testosterone might be by inversion of the 17-OH group. (Supported by E.R.Squibb & Sons and USPHS grant A-3124 Endo.)

Biochemistry

7

ASSOCIATION BETWEEN TESTOSTERONE AND AMINO ACIDS. Denis Abelson and Corrine B. Depatie Yale Univ. School of Med., New Haven, Conn. (intr. by C.N.H. Long)

In view of the possibility that steroid-protein interactions may depend in part on associations between steroids and individual amino acids, solubility studies were performed, using testosterone as a model steroid. The solubility of the latter was determined in solutions of various L-amino acids. Experiments were generally carried out at 31° C. in phosphate buffer at pH7, and at amino acid concentrations of 0.01 to 0.05M. In the case of the poorly soluble L-tyrosine, an unbuffered solution of the ethyl ester hydrochloride was used. After shaking until equilibrium was reached the solutions were filtered, and the testosterone extracted with chloroform. The chloroform extract was dried, and the residue dissolved in ethanol. The concentration of steroid was determined by ultra-violet spectrophotometry. Among 18 amino acids studied, the solubility of testosterone was increased in two - L-tryptophan and L-tyrosine ethyl ester. A small and questionable increase was found in 8 other amino acids. Assuming a 1:1 ratio of interactants, the apparent K for the association of testosterone with L-tryptophan is about $14M^{-1}$, and with L-tyrosine ethyl ester about $6M^{-1}$. It is concluded that testosterone can form associations with L-tryptophan and L-tyrosine ethyl ester.

Biochemistry

9

NON-UTILIZATION OF MEVALONIC ACID IN STEROIDOGENESIS BY TESTIS TISSUE. Kenneth Savard, Morton Goldweber* and Joseph W. Goldzieher*. University of Miami School of Medicine, Miami, Fla. and Dept. Endocrinology, Southwest Foundation for Research and Education, San Antonio, Tex.

DL-Mevalonic acid-2-C^{14} (MVA-C^{14}, 10 uc.) was incubated with 3.8 g. of fresh dog testis slices in 10 ml. of Krebs-Ringer bicarbonate medium, pH 7.1, at 37° in O_2-CO_2 for 3 hrs. with and without added human chorionic gonadotropin (APL, Ayerst). In a second experiment, two intact stallion testes were perfused for 12 hours with 1.4 l. of defibrinated horse blood containing DL-MVA-C^{14} (240 uc.); in the course of this perfusion 10 mg. of androgen (measured by bioassay) were formed. At the completion of the incubations and perfusion the following steroids were added as carriers: progesterone, 17-hydroxyprogesterone, 4-androstene-3,17-dione and testosterone. All steroids were recovered devoid of significant radioactivity following rigid purification steps. A small but significant amount of radioactivity was in the non-polar lipids in which fatty acid and cholesterol of low specific activity (20 c./m./mg.) were found. These results suggest that intact testis tissue, while capable of utilizing acetate-C^{14} for steroid synthesis, (Savard and Goldzieher (1960)) cannot utilize exogenous mevalonic acid. (Supported in part by Grants C-4004 and A-767, U. S. Public Health Service.)

XUM

114. ENDOCRINES–Sex Hormones–Estrogens

1 *Physiology*

EFFECT OF STEROID SUPPLEMENTATION ON LIFE SPAN OF THE FEMALE RAT. G. M. Emerson and J.D. Emerson (intr. by John M. Bruhn) Dept. of Physiol., Univ. of Ala. Med. Center, Birmingham.

Experimentally and clinically low dosage supplementation of androgen-estrogen and/or glucocorticoid appears to increase vigor of old mammals but not to increase life span markedly. In this experiment 2 treatments were given at dosages calculated to give tissue levels, of the steroids involved, above young adult levels. Rx 1: methyltestosterone 0.75 mg., diethylstilbestrol 0.05 mg., cortisone 0.15 mg., DOCA 0.15 mg./ rat, 1 injection every 2 days. Rx 2: methyltestosterone 0.75 mg., diethylstilbestrol 0.05 mg., cortisone 0.3 mg., DOCA 0.3 mg./rat, 2X/week.

Group	No. Rats	Age Days Onset Rx	Survival on Rx (Days)
Rx 1	8	946	202.0±24.2
Control 1	7	947	106.6±19.2
Difference		-1	95.4±30.9
Rx 2	9	774	229.3±27.7
Control 2	16	774	237.4±29.5
Difference		0	-8.1±40.5

All means ± their standard errors.

2 *Biochemistry*

STUDIES OF A SOLUBLE ENZYME SYSTEM FROM HUMAN PLACENTAL MICROSOMES CONVERTING TESTOSTERONE-4-C14 TO C14-ESTRADIOL-17β. Nina Hollander* and V. P. Hollander. Univ. of Va. School of Med., Charlottesville, Virginia.

Conditions for the quantitative conversion of testosterone -4-C14 to C14-estradiol-17β by human placental microsomes in 0.05 M phosphate buffer are pH 7.3, the presence of Na^+ 0.03 to 0.3 M, K^+ 0.1 M, and Mg^{++} 0.01 M. The enzyme system was stable when kept overnight at room temperature at pH 7, and at -20° for several months. It was stable when kept 24 hours at 0° at pH 9 but not at pH 3. The enzyme system could be solubilized by treatment in a 10 KC Raytheon sonic oscillator for 10-30 minutes, but not by treatment with desoxycholate, digitonin, RNAase, lipase, lyophilization, butanol, or acetone. The activity of the solubilized system could be precipitated with ammonium sulfate at pH 7 when saturation was less than 33%. Extent of conversion was proportional to the amount of protein, possibly indicating a bound unknown cofactor limiting the reaction. Either TPNH or DPNH was essential to the conversion, and could not be replaced by N-benzyldihydronicotinamide, reduced glutathione, cysteine, dehydroascorbic acid, or H_2O_2. Catalase had no effect. The system could be completely inhibited by FMN or FAD at 10^{-3} M.

3 *Biochemistry*

INTERACTION OF ESTROGEN SULFATES WITH B_6-ENZYMES: EVIDENCE FOR PHYSIOLOGICAL SIGNIFICANCE. M. Mason and E. C. Gullekson.* Dept. of Biological Chemistry, Univ. of Michigan, Ann Arbor, Michigan.

Earlier studies from this laboratory (J. Amer. Chem. Soc., 81, 1517 (1959), J. Biol. Chem., in press) have shown that very low concentrations of estrone sulfate and of the disulfate esters of estradiol and diethylstilbestrol inhibit kynurenine transaminase and phosphorylase a, most probably by interfering with their association with pyridoxal phosphate. In the present study, evidence concerning the physiological significance of this interaction was sought. In one line of investigation, the inhibitory potencies of a variety of sulfate esters, including those of allenolic acid, bisdehydrodoisynolic acid, dienestrol and a variety of stilbestrol analogues, were found to have a positive correlation with the estrogenic potencies of the parent phenols. In another, profound sex differences in the development of kidney kynurenine transaminase were found, the level in the mature males being about 3 times that of the mature females. The effects of ovariectomy and estrogen supplementation on these levels implicate the estrogens as causitive agents in retarding the development of the enzyme in the females. Similar sex differences were found with kynureninase, another B_6-enzyme which is inhibited by estrogen sulfates. (Supported by a grant from USPHS.)

4 *Biochemistry*

CONJUGATION OF ESTROGENS BY THE HUMAN FETUS. Mortimer Levitz, George P. Condon* and Joseph Dancis*. Depts. of Obstetrics and Gynecology and Pediatrics, New York Univ.-Bellevue Med. Center, New York City.

Two fetuses of approximately 3 and 5 months gestation were studied. Following delivery by hysterotomy, 30 μg. of estrone-16-C14 (3 x 10^4 c.p.m./μg.) in saline was injected into the umbilical vein. After 45 minutes, during which period the heart continued to beat but the fetus did not breathe, the fetus was homogenized and analyzed. 67% of the radioactivity was ether-soluble and 33% was water-soluble. In the ether-soluble fraction, only estrone (67%) and estradiol (4%) could be identified (by chromatography and reverse isotope dilution). The water-soluble fraction resisted hydrolysis by β-glucuronidase. Paper chromatography revealed one radioactive band at the same R_f as estrone sulfate. Following elution, the conjugate was further identified as the sulfate by hydrolysis with phenolsulfatase (87-94%) with inhibition by phosphate. The steroid fraction consisted of estrone (90%) and estradiol (10%).

5 *Biochemistry*

FATE OF STEROID ESTROGENS IN TARGET TISSUES. J. W. Flesher, G. N. Gupta,* H. I. Jacobson, and E. V. Jensen. Ben May Laboratory, Univ. of Chicago, Chicago, Ill.

Development of a procedure for preparing and handling steroid estrogens labeled with carrier-free tritium and of an assay method for tritium in animal tissues has made possible the determination of the amount and chemical nature of the steroid present in rat tissues following administration of physiological doses of estrogenic hormones. Uterus, vagina and pituitary were found to differ from other tissues, such as liver, kidney, muscle, adrenal, bone and blood, in their steroid incorporation pattern in that these accessory sex tissues continue to incorporate and retain steroid at a time when the level in the other tissues is markedly decreasing. Following the administration of tritiated estradiol, the radioactive material present in uterus is nearly all free estradiol, in sharp contrast to liver where binding to protein, formation of water-soluble conjugate and conversion to other steroids takes place.

6 *Biochemistry*

INFLUENCE OF ESTROGENS ON PHOSPHOLIPID METABOLISM IN RAT UTERINE SEGMENTS. Yoshio Aizawa* and Gerald C. Mueller. McArdle Memorial Laboratory, Univ. of Wis. School of Medicine, Madison, Wis.

In response to a single dose of estradiol the level of ethanolamine, choline, and inositol phospholipids increased as much as 100% per cell in the first 6 hours. The accumulation of phospholipids appears to reflect an increased rate of synthesis since P^{32} incorporation into the various phospholipid fractions of surviving estrogen-stimulated segments incubated in Eagle's tissue culture medium was accelerated to 3 times the control level in this period. The direct addition of .05 ugm. of estradiol per 2.0 ml. of tissue culture medium resulted in a 30 to 50% stimulation of P^{32} incorporation into surviving segments from control animals. While estrone and estriol were also active at this level, diethylstilbesterol, progesterone, cortisol and desoxycorticosterone were not effective in this system. The experiments reveal phospholipid metabolism to be a very early and sensitive indicator of estrogen action in the rat uterus.

7 *Biochemistry*

MICROBIOLOGICAL HYDROXYLATION (C-16) OF ESTRONE, ESTRADIOL-3,17α AND ESTRADIOL-3,17β.
E. F. Stimmel, T. E. Bucknell* and Vessa Notchev*
Rees-Stealy Clinic Research Found., San Diego, Calif.

Since several species of Streptomyces have been employed successfully for 16α-Hydroxylation of neutral steroids (Fried, J.; Perlman, D.; Langlykke, A. F.; and Titus, E. O.; U.S. Patent 2,855,343, Oct. 7, 1958) it seemed of interest to pursue similar studies with the phenolic steroids. Accordingly, we have incubated aerobically each of the natural estrogens, estrone, estradiol-3,17α, and estradiol-3,17β with proliferating cultures of several species of Streptomyces (S. bikiniensis, S. rimosus, S. griseus). Incubation of estrone with S. bikiniensis yielded an 11-ketolic estrogen which by sodium borohydride reduction yielded estriol-3,16α,17β. In both Zaffaroni and Bush paper chromatographic systems the 11-ketol resembled synthetic 16α-Hydroxyestrone. Yields have not exceeded 10%. Similar incubations of estradiol-3,17α and estradiol-3,17β have yielded the C-16 hydroxylated glycols estriol-3,16β,17α and estriol-3, 16α,17β respectively.

115. ENDOCRINES—Thyroid Gland

1 *Physiology*

FORMATION OF IODOTYROSINES IN THYROID MITOCHONDRIA AND MICROSOMES. Winton Tong* and I. L. Chaikoff. Dept. of Physiology, Univ. of California, Berkeley, Calif.

Isolated thyroid mitochondria and microsomes form mainly labeled monoiodotyrosine (MIT) with only traces of diiodotyrosine (DIT) when incubated with I^{131}. The ratio MIT-I^{131}/DIT-I^{131} was always 7 to 10. To observe whether or not this finding reflects the normal situation in intact thyroids, cell fractions were isolated by differential centrifugation from sheep thyroid tissue slices previously incubated for 15, 30, 60 and 120 min. with I^{131}. The mitochondrial, microsomal and soluble fractions were hydrolyzed with pancreatin and analyzed by paper chromatography. The data in the table show

Incubation time (min.)	MIT-I^{131}/DIT-I^{131} observed in:		
	Soluble fraction	Mitochondria	Microsomes
15	3.5	10.0	14.6
30	4.0	11.4	15.4
60	3.1	6.9	9.5
120	2.3	3.4	4.6

that the ratio of newly formed MIT-I^{131} to DIT-I^{131} was at all intervals higher in the particulate matter than in the soluble fraction. These findings indicate that thyroid mitochondria and microsomes, whether incubated in tissue slices or isolated and incubated separately, form MIT readily, but convert MIT to DIT more slowly.

2 *Physiology*

RESERPINE AND THYROID ACTIVITY IN THE PIGEON. B. N. Premachandra and C. W. Turner. (intr. by B. A. Westfall). Dept. of Dairy Husbandry, Univ. of Missouri, Columbia.

Several studies are being conducted in poultry in search of an appropriate tranquilizer because of its laboratory and commercial importance. Recently, there has been some evidence to indicate that reserpine inhibits thyroid activity under experimental conditions but the controlled data in poultry has been extremely limited, if not non-existent. Thyroid activity was estimated in the pigeons (maintained under constant and uniform environment) by the determination of individual thyroxine secretion rate using replacement techniques. Reserpine administered subcutaneously at levels 5, 10 and 15 ug./100 g. body weight had no effect on thyroxine secretion rate as compared to vehicle injected controls. Levels above 15 ug./100 g. were increasingly toxic and thyroxine secretion rate was depressed at all higher levels. The depression of thyroxine secretion rate was, however, accompanied by decreased feed intake. Body weight losses followed a parallel trend with reducing thyroxine secretion rates. At non-toxic levels administration of reserpine did not affect thyroxine secretion rate. It is therefore suggested that thyroid inhibition observed at higher levels of reserpine is very likely due to effect of inanition since thyroid inhibition was not observed at those levels where there was no decreased feed intake.

3 *Physiology*

CHROMATOGRAPHY OF IODINATED AMINO ACIDS ON SULFONATED POLYSTYRENE RESINS. Samuel R. Lerner, (intr. by J.R. Elliott), Radioisotope Service, Veterans Admin. Hosp., Houston and Biochemistry Dept., Baylor University College of Medicine.

A method for separation of the iodinated amino acids has been developed utilizing the excellent separations achieved by elution analysis (Moore, Spackman & Stein, Anal. Chem. 30:1185 & 1191, 1958). A continuous recording of the optical density at 254 mμ is provided. A 15 cm column of Dowex 50X4 resin, 30-40 μ particles, gives excellent separation. Dowex 50X2 is satisfactory; IR-120X8 gives poor resolution of the iodinated thyronines. The effluent, at 25 ml per hr, passes through a flow cell of silica, where the absorbance is measured, then to a fraction collector. The iodinated tyrosines are separated at pH 3.5-4.5 in a 30% ethanol 0.2N ammonium formate buffer in sequence: tyr, phe, mit, try, dit. After 7 hrs (175 ml), the pH 9.2 buffer is started and 3,5-diiodothyronine, thyroxine and triiodothyronine elute in that order. This fractionation is complete in 10 hrs (250 ml) and 0.2N NaOH removes little additional material. The column is regenerated by the pH 4.5 buffer and is then ready for re-use. The UV absorbance recording can be calibrated so that the area under the curve yields quantitative analyses. Over 95% of the added radioactivity is recovered. Application of the method to thyroid gland fractionation will be discussed.

4 *Physiology*

EFFECT OF THYROXINE PRETREATMENT ON UPTAKE AND ORGANIC BINDING OF I^{131} BY INCUBATED GUINEA PIG THYROID SEGMENTS. C. Fortier and W. J. Schindler.* Blue Bird Neuroendocrinol. Res. Labs. and Dept. of Physiol., Baylor U. Coll. of Med., Houston, Texas.

Incubation of guinea pig thyroid segments in a medium containing I^{131} revealed that both uptake and organic binding of I^{131} (as determined by TCA precipitation) responded quantitatively to TSH. The PBI^{131}/tissue weight ratio provided a composite indicator of the two effects, more sensitive than either. Radiochromatography of non-hydrolysed tissue extracts confirmed results of TCA precipitation with respect to distribution of organic and inorganic I^{131}, and effect of TSH on protein binding. MIT and DIT, the only iodinated amino-acids detected in pancreatin hydrolysates, respectively accounted for 50 and 10% of the total radioactivity of non-stimulated samples. TSH was found to accelerate iodination of MIT into DIT. To assess effect of prior blockade of endogenous TSH secretion, glands were collected from donors pretreated with DL-thyroxine. Within 8 days of treatment, basal in vitro uptake and protein binding of I^{131} were depressed to 50% of normal. Responsitivity to TSH evidenced a two-threefold increase after 2 days, and slowly declined thereafter. These results, supplemented by evidence of a linear log dose-response relationship over a wide range, provide the basis for a simple and accurate TSH assay. (Supported by Air Force Contract No. AF 49(638)-384.)

5 *Physiology*

EFFECT OF AGE AND OBESITY ON BASAL OXYGEN CONSUMPTION OF WOMEN AS RELATED TO BODY FLUID COMPARTMENTS. Saul P. Baker, Nathan W. Shock and Arthur H. Norris.* Gerontology Branch, Nat'l. Heart Inst., Bethesda, and Balt. City Hosps., Balt., Md.

Basal oxygen consumption per unit surface area in both men and women classically decreases with age. In men, no decrease occurs if expressed per unit total body water or intracellular water. Specific indices of thyroid function are reportedly higher in women as compared to men of the same age group, although classical basal oxygen consumption per square meter is lower in women. Basal oxygen consumption was, therefore, determined in 58 euthyroid women, aged 20 to 96. Total body water (antipyrine) and extracellular water (thiocyanate) were determined individually, the intracellular compartment being obtained by difference. The classical agewise decrement of basal oxygen consumption per square meter was confirmed, the older obese women falling at the lower end of the distribution. However, no significant regression with age or obesity was found when basal oxygen consumption was expressed in terms of total body water or intracellular water. It is suggested that age and obesity have no influence on basal oxygen consumption of women if this is expressed in terms of total body water, or more precisely, intracellular water, the latter being taken as an approximation of functional cell or protoplasmic mass.

XUM

6 *Physiology*

GENETIC SELECTION AND THYROID ACTIVITY. Joseph H. Sunder (intr. by Campbell Moses.) Addison H. Gibson Laboratory, U. of Pittsburgh School of Med., Pittsburgh, Pa.

Shohlee et al. 1955 (Science 34: 572) and others have shown that certain thyroid responses are under genetic control. To study the effect of selection on I^{131} retention the following experiment was performed. An F_2 generation of 23 ♂ and 21 ♀ was bred from a single pair of Carworth albino rats. I^{131} retentions (24-hour) were obtained in these 44 rats and pairs with high retention and low retention were bred for 5 generations. Unselected animals from the original colony were similarly bred. The mean F_2 I^{131} retention value was $21.2 \pm 6.1\%$.

	S_1 %	S_2 %	S_3 %	S_4 %	S_5 %
High	35.7 ± 4.2	41.2 ± 3.4	43.8 ± 2.7	44.8 ± 1.2	45.2 ± 0.8
Low	11.4 ± 2.8	8.7 ± 1.5	7.9 ± 1.1	7.2 ± 0.7	7.1 ± 0.5
Unselected	22.8 ± 5.3	22.1 ± 3.9	22.1 ± 3.9	23.2 ± 5.8	23.0 ± 6.6

After 5 generations both the high and low crosses produced no living pups although vaginal smears were normal. Autopsy 15 days after mating revealed abnormal feti in utero. Eight of 10 S_5 low retention ♀ delivered normal pups when thyroxine was added to the drinking water. These studies indicate that I^{131} retention is partially under genetic control.

7 *Physiology*

PROTEOLYSIS OF PHOSPHATE-PURIFIED THYROGLOBULIN. A.D. Litonjua (intr. by N. Bucher.) Mass. General Hospital, Boston, Massachusetts.

An acetone-purified thyroid protease free of thyroglobulin has slow hydrolytic action on thyroglobulin prepared from the 41-48% phosphate fraction of a saline extract of beef thyroids. The thyroglobulin also hydrolyzes itself readily at 37° C. Both the protease and the thyroglobulin break down hemoglobin readily. Degradation is optimal at pH 3-3.5. Heating the thyroglobulin at 75-100° C prir to the 37° C incubation completely abolished the autohydrolysis, but hemoglobin breakdown was not much impaired. Fractionation procedures of the thyroglobulin have resulted in a product with higher proteolytic activity against hemoglobin, but when added to the original thyroglobulin the slow proteolytic property of the acetone-purified protease is simulated. This suggests that fractionation has caused loss of some crucial thyroglobulin-protease bonding necessary for immediate action, or denaturation of the enzyme with a decrease in potency towards thyroglobulin, but not toward hemoglobin. (Supported by Grant #A-1880 from the U.S. Public Health Service.)

8 *Pathology*

STUDIES ON A FORM OF METABOLIC THYROIDITIS IN THE HAMSTER. R.H. Follis, Jr. V.A. Cent. Lab. Anat. Path. and Res., AFIP., Washington, D.C.

When excessive amounts of iodine (10 mg. KI intraperitoneally) are administered to hamsters which have thyroid glands made hyperplastic by an iodine deficient diet or administration of goiterogens, an acute inflammatory reaction rapidly develops. (Follis, Proc. Soc. Exp. Biol. Med., 102:425, 1959). Present experiments were designed to determine levels of iodine administration which induce the change. Thyroid hyperplasia was produced in hamsters by the administration of a low iodine diet containing .3% thiouracil. After 6 to 8 weeks on this regimen, when epithelial proliferation had become extreme, the goitrogen was removed from the diet and graded amounts of KI (ranging from .001 to 10 mg.) were administered intraperitoneally daily to different groups. All animals were sacrificed after 6 days of such treatment; their thyroid glands were examined microscopically. No inflammation was found in controls; colloid had reaccumulated in excess. At the level of .001 mg. only occasional desquamated cells and leukocytes were found within the follicles; somewhat less colloid had reaccumulated. A severe acute inflammatory reaction was found in those animals which had received KI in excess of .01 mg. Less colloid accumulated at the higher dose levels.

116. ENDOCRINES–Thyroid Gland–Iodine Metabolism

1 *Physiology*

IN VITRO UPTAKE OF I^{131} BY MAMMARY TISSUE. M. Maqsood* and E. P. Reineke, Department of Physiology and Pharmacology, Michigan State University, East Lansing.

In vitro uptake of I^{131} (I*) by lactating mammary slices incubated at 37°C for 6 hr. was 10-15 times that of liver, muscle or boiled mammary slices. I* uptake by lactating mammary slices increased progressively up to the 6th hr. of incubation, but remained constant in boiled tissue after the first ½ hr. Uptake was greatly reduced by the addition of thiouracil and blocked by KSCN, NaCN or incubation under N_2. I* uptake was increased to about twice the level observed in fresh mammary tissue, but not in liver or muscle, by storage for 24 hr. in Ringer-phosphate at 7°C, and continued high for 20 days' storage. Storage of liver or muscle with mammary tissue had no effect on I* uptake by the former. Bacterial contamination was ruled out as a factor by the non-effect of penicillin on I* uptake in fresh and stored tissue, and histological observation of intact alveolar cells after extended storage. Storage for 1-4 days at 37°C (with penicillin) resulted in a progressive decline in uptake when subsequently incubated with I* for 6 hr. I* uptake by fresh mammary tissue was low during early pregnancy and increased sharply at 15-20 days. It rose further during lactation and declined to minimal values after 10 days' involution. Significant I* uptake also occurred in mammary tissue of mouse, guinea pig and rabbit.

2 *Physiology*

EFFECT OF GRADED DOSES OF PROPYLTHIOURACIL(PTU) ON BIOSYNTHESIS OF THYROID HORMONE. S. Iino and T. Yamada (intr. by W. D. Blake) U. Ore. Med. Sch., Portland

PTU inhibits not only the initial monoiodination of tyrosine but also both the diiodination of tyrosyl radicles and their coupling to form T_3 and T_4. However, the effect of large doses of PTU on thyroid biosynthesis has not been studied in detail. Graded doses of PTU (0.001 - 60 mg.) were administered subcutaneously once daily to rats for 3 days; 20 - 100 μc I^{131} were given immediately following last dose PTU. Twenty-four hours later, thyroids were counted, homogenized, digested with pancreatin, then chromatographed in BuOH-Ac-H_2O. Relative amounts of DIT and of $T_3 + T_4$ decreased with increasing doses of PTU; no DIT nor $T_3 + T_4$ were formed at concentrations >0.1 mg. The relative amount of MIT increased with the dose of PTU, attained its maximum at a conc. of 5 mg., then declined. Even with 60 mg. PTU, MIT still accounted for >10% of total thyroidal radioactivity. The absolute amount of I^- declined sharply with doses >0.01 mg. Formation of DIT from MIT is thus inhibited by very small doses of PTU whereas organic binding of I^- into MIT is not completely blocked even with relatively huge doses.

3 *Physiology*

KINETICS OF RADIOIODINE UPTAKE IN MICE AND RATS. S. H. Wollman and F. E. Reed*. National Cancer Inst., Bethesda, Md.

Thyroidal radioiodide and protein-bound radioiodine and serum radioiodide concentrations were measured at 3, 6, 10 and 16 min. after I. P. injection of radioiodide. The ratio of the I^{131} bound per min. in the thyroid to the thyroid radioiodide content (K_b) was constant for normal animals and ranged from 0.15 to 1.7 depending on the diet. In hypophysectomized mice K_b decreased with time after injection and approached 0.01 at 1 hour. The ratio of the radioiodide concentrations in thyroid and serum (T/S) was much lower when organic binding was permitted than when blocked. The time interval required to attain a steady state T/S was much less when binding was permitted than when blocked. This seemed largely due to the high values of K_b. Thyroid clearances of serum radioiodide were constant during runs and were 20, 40% higher than one-way clearances determined in duplicate mice or rats, resp. Analysis indicates that the constant clearances observed are related to the rapid radioiodide equilibration: for clearances are expected to start at maximum value and decrease to the steady state value as the thyroid radioiodide concentration increases to steady state T/S. Data suggested that when K_b was large enough the fraction of the thyroid radioiodide transferred to blood per minute was larger when binding was permitted than when blocked.

4 *Physiology*

IODINE CONTAINING PARTICLES IN THYROID TISSUE. David W. E. Smith* and J. E. Rall. National Institute of Arthritis and Metabolic Diseases. Bethesda, Maryland.

Normal thyroid tissue contains a small amount of iodine in subcellular particles. A method has been developed by which many particulate fractions can be prepared by centrifuging aliquots of homogenized tissue at increasing forces. The iodine containing particles occur in three centrifugally separable fractions. Two of these fractions are collected within the range of forces usually used to isolate mitochondria, and the other is in the microsomal range. The iodine containing particles have been studied for iodinating activity using a system containing I^{131} and FAD at pH 9 to 10. The fraction containing the smallest iodinated particles is most active in incorporating I^{131} into a TCA precipitable form. In a transplantable rat thyroid tumor which incorporates substantial amounts of I^{131} into particles it has been shown that the iodinated particles occur in three fractions similar to those in normal thyroid tissue. Sucrose gradient centrifugation confirms the existence of at least two groups of iodinated particles and indicates that the large particles are more dense than the smaller ones.

5 *Physiology*

CHEMICAL IDENTIFICATION OF IODINE-CONTAINING COMPOUNDS IN HUMAN SERUM. Tsuyoshi Kono* and E. B. Astwood. New England Center Hosp. and Tufts Univ. School of Med., Boston, Mass.

5 to 10 ml. of human serum were extracted with acid butanol containing 0.1 N H_2SO_4 which was then made alkaline and evaporated in vacuo to 1 ml., and filtered. To the filtrate and washings an equal volume of $CHCl_3$ was added and this mixture extracted with 2 N NH_4OH. The ammonia solutions were evaporated in vacuo. The residue was dissolved in 1% NH_4OH in methanol and chromatographed in n-BuOH-HOAc-H_2O (75:10:15) and in n-BuOH-EtOH-2 N NH_4OH (5:1:2 or 5:2:1) systems. The papers were stained with a ceric sulfate-arsenious acid reagent, followed by aniline in acetone and neutralized with $NaHCO_3$. Chloride simulated iodine compounds on chromatograms. Thiouracil, its disulfide and propylthiouracil were found to react catalytically and simulated iodotyrosines or iodothyronines on chromatograms. In 21 sera from normal persons and patients with simple goiter thyroxine and inorganic iodide were regularly identified. Iodotyrosines have not yet been found.

6 *Biochemistry*

GOITER IN MICHIGAN. Isidor Greenwald. New York University College of Medicine, New York, N. Y.

Goiter was prevalent in Detroit in 1800 and 1810. Examination of many varied sources failed to disclose subsequent mention of goiter anywhere in Michigan for more than 50 years (Ann Arbor, Medina, 1866; Grand Rapids, 1878; Kalamazoo, 1882; Detroit, 1893). A travelling quack boasted of 11 cases of goiter that he had treated in the first six months of 1873. (Advertisements in Gratoit, Midland, Saginaw). Advertisements of patent medicines and by quacks continued for many years but the next clear indications of goiter in Michigan that I found were in 1912 (Escanaba and Detroit). A few years later, such advertisements were more numerous. Hospital reports indicate an increase in the proportion of goiters that began about 1908 and continued for many years. Are not such increases as significant as the decreases that followed the introduction of iodized salt? The three different accounts by Marine of a severe outbreak of goiter in sheep, stopped by the use of a local salt containing iodine are unauthenticated. Neither the severe outbreak nor the production of an iodine-containing salt can be verified from the public records, which appear to be quite adequate.

7 *Biochemistry*

THE PHOTOINDUCED IODINATION OF SOME PROTEINS. M. Soodak and F. Maloof*. Grad. Dept. of Biochemistry, Brandeis Univ., Waltham, Mass.; Dept. of Med., Mass. Gen. Hosp., Boston, Mass.

The stimulation of the formation of protein bound monoiodotyrosine in thyroid particulate preparations by the flavins was shown to be, for the most part, a photoinduced stimulation (Fed. Proceed. 18, 328, 1959). This led to the development of the following photochemical system for iodination. The incubation mixture contains 200 μmoles FMN, 150 μmoles Tris (pH 7.4), from 2-10 mgs. of a protein, and carrier-free I^{131} in a final volume of 1.5 ml. The reaction is carried out in air in pyrex test tubes (18 x 150) at 37°C with shaking for 15 minutes. A long wave length ultraviolet lamp (Black-Ray) was placed at a distance of 15 cms. from the bottom of the tubes. Only bovine serum albumin (BSA) and lysozyme of a series of eight crystalline proteins are iodinated. Protein bound monoiodotyrosine is formed as the sole product and in amounts comparable to that formed in the usual enzymatic incubation of thyroid particulate preparations. The photoinduced iodination of BSA is strongly inhibited by either catalase or by running the reaction under nitrogen. Hydrogen peroxide is probably the major oxidant for the iodide. The reasons for the interest specificity of the protein acceptor is being investigated.

8 *Biochemistry*

THE MECHANISM OF IODINATION REACTIONS IN THYROID GLANDS. Nicholas M. Alexander (intr. by Donald L. Buchanan.) Radioisotope Service, V. A. Hosp., West Haven, and Dept. of Biochemistry, Yale Univ., New Haven, Conn.

Previous studies from this laboratory showed that inorganic iodide is catalytically oxidized by H_2O_2 and an enzyme, iodide peroxidase, before it is incorporated into tyrosine and protein by thyroid and salivary homogenates. It has been found that several other naturally occurring biological oxidants or electron carriers cannot replace H_2O_2, although others have shown that flavin cofactors stimulate iodide utilization by thyroid preparations. The finding that catalase or anaerobiosis prevent flavin activation indicates that this activation is due to the production of H_2O_2 by autoxidation of the flavin. The peroxidases of salivary and thyroid glands are not identical because salivary homogenates oxidize bromide and to a slight extent incorporate it into tyrosine, but thyroid homogenates will not. Neither tissue oxidizes chloride nor fluoride. Histidine is iodinated by thyroid and salivary extracts but tyrosine is overwhelmingly iodinated when both amino acids are present. Although iodohistidine has been isolated from thyroid glands, it has not previously been biosynthesized in vitro. Under conditions where tyrosine is readily iodinated, the possible thyroxine precursors, thyronine, di- and triiodothyronine, are not iodinated by rat or human thyroid extracts.

117. ENDOCRINES—Thyroid Gland—Thyroxine

1 *Physiology*

THYROID HORMONE INDUCED COPPER DISCHARGE IN THE TADPOLE. H.J. Lipner, Tony J. Miller* and Mary C. Muntz*, Div. of Physiology, Florida State University, Tallahassee, Florida.

An inverse relationship has been found to exist between the copper concentration of some rat tissues and the metabolic level of these tissue (Lipner, et.al. The Physiol. 2, 1959). Excretion of copper was examined in tadpoles in which metamorphosis and elevation of metabolism was induced with triiodothyronine (T_3) or thyroxine (T_4). Bufo tadpoles were immersed in Cu^{64} solution, with carrier copper not exceeding 10^{-6}M, for 12 hours. The tadpoles were washed in 0.01M NaCl solution, immersed in $1x10^{-7}$M T_3, $3x10^{-7}$M T_4, $5x10^{-9}$M Propionic acid analogue of T_3(T_3 prop) $1x10^{-4}$M EDTA, $1x10^{-7}$M EDTA or $1x10^{-6}$M DNP, and counted at time of immersion and at 4 hour intervals. The T_3 treatment was repeated in Rana pipiens at $1x10^{-7}$M without effect and at $1x10^{-6}$M at which concentration a response was obtained. T_3,T_4, and T_3 prop caused a loss of Cu^{64} in Bufo within 12-20 hours and at 44 hours was reduced to about 20% of the initial level as compared with the controls which were 90% of the initial level. EDTA at 10^{-4}M concentration was effective but at 10^{-7}M was ineffective at causing copper loss. DNP resulted in a short lived loss of Cu^{64} followed by a reaccumulation of Cu^{64}. The data suggest that in the tadpole as in the rat increased metabolic activity is accompanied by the loss of copper. (Supported by a grant from PHS).

XUM

2 *Physiology*

PREVENTION OF TAPAZOLE-GOITRE AND THYROIDAL I^{131} RELEASE WITH THYROXINE WITHOUT ALTERATION OF PBI. Clark E. Grosvenor with technical assistance of Anthony Whitehead (Intr. by D.A. Brody) Department of Physiology, University of Tennessee, Memphis.

Adult female rats maintained with Purina Lab. Chow were bilaterally ovariectomized and individual thyroid secretion rates (TSR) determined by the thyroxine replacement technique. A mean TSR of .40ug/100g/day l-thyroxine was obtained. Two weeks later the same rats were injected daily for 2 weeks with either 2.5mg/100g/day Tapazole or with Tapazole together with the individual TSR amount of thyroxine. The dose of Tapazole used effectively prevented I^{131} uptake by the thyroid and induced significant thyroid enlargement. Such enlargement was prevented by concurrent administration of TSR amount of thyroxine. Plasma PBI levels, however, were not significantly altered from those of untreated or Tapazole-treated castrate rats. Sustained blockade of thyroidal I^{131} release occurred following daily injection of TSR amount of thyroxine again without alteration of PBI. It would appear that the effect of the TSR amount of thyroxine in preventing thyroidal I^{131} release or goitre in Tapazole-treated castrate female rats is achieved without elevation of plasma PBI. (This investigation was supported by a Senior Research Fellowship (SF-365) from the Public Health Service.)

3 *Physiology*

INFLUENCE OF pH AND AMMONIA ON THYROXINE MAINTENANCE OF KIDNEY METABOLISM. R. H. Lindsay,* R. W. Hanson* and S. B. Barker. University of Ala. Medical Center, Birmingham, Alabama

Long term incubation experiments with rat kidney slices at 37° in an L-proline medium with added thyroxine (T_4) gave a net production of glutamate, glucose and NH_3 with an increase in pH. The concentrations of these compounds were higher and O_2 consumption better maintained in the presence of T_4 than in its absence. With L-glutamate as substrate, glucose levels were considerably lower than with proline. O_2 consumption dropped more rapidly although better maintenance occurred with T_4. In this medium NH_3 was formed at a rate comparable to that in proline for 9 hr but was less after 12 hr. The pH increased from the initial 7.5 to 8.3 in 3 hr and continued more slowly to 8.7 after 12 hr. With proline, pH remained steady with no observed change until 6 hr. At that time it was 7.8 and reached a peak of 8.0 at 12 hours. In the proline medium, raising the starting pH to 8.5 had no effect on O_2 consumption. Adding NH_3 initially in the quantities usually formed during a 12 hr incubation produced a slight decrease in O_2 uptake, glucose accumulation and production of additional NH_3. A combination of high starting NH_3 and elevated pH resulted in a pronounced drop in O_2 consumption plus lower levels of glucose and NH_3. Glutamate results were similar, including a rapid pH rise during incubation.

4 *Physiology*

THE EFFECT OF THYROXINE ON ITS OWN TURNOVER TIME. C. W. Pipes, B. N. Premachandra and C. W. Turner (intr. by Homer E. Dale). Dept. of Dairy Husbandry, Univ. of Missouri, Columbia.

Since thyroxine increases the metabolic rate of practically all cells of the body, it is not surprising that increased levels of thyroxine reduce the turnover time ($t_{\frac{1}{2}}$) of I^{131}-tagged l-thyroxine. In female rats the normal $t_{\frac{1}{2}}$ of thyroxine was 23.4 hours while in those equilibrated with 2.5 micrograms of thyroxine the $t_{\frac{1}{2}}$ was reduced to 9.97 hours. At the 3.5 microgram level an apparent plateauing effect occurred since the $t_{\frac{1}{2}}$ was 10.84 hours. In dairy cattle the normal $t_{\frac{1}{2}}$ of radiothyroxine averaged 2.5 days in 15 observations. When thyroxine was injected at 50% above the normal secretion rate, the $t_{\frac{1}{2}}$ was reduced to 1.0 days. At 100% above the normal secretion the $t_{\frac{1}{2}}$ was found to be 0.8 days. It is postulated that at high levels of thyroxine secretion (hyperthyroidism) or administration (thyroprotein feeding), not only the turnover of thyroxine is decreased, but other hormones and vitamins as well.

Aided in part by a grant from the U.S. Atomic Energy Commission (Contract No. AT(11-1)-301).

5 *Physiology*

INTESTINAL METABOLISM OF THYROXINE. S.L. Jones,* L. Wayne,* AND L. Van Middlesworth. Dept. of Physiol. Univ. of Tenn., Memphis.

A major fraction of the circulating thyroxine of the rat is secreted into the intestine as biliary thyroxine glucuronide; the conjugate is poorly reabsorbed but is not found in the feces (Albert and Keating 1952, Taurog 1952). Therefore, the site of hydrolysis of the conjugate may determine the thyroxin requirement. Rats were injected daily with I^{131}-thyroxine tracer and after seven days the I^{131} compounds in the intestinal contents were extracted and separated by paper chromatography. The I^{131} in the proximal 1/3 of the small intestine was primarily thyroxine glucuronide. The remainder of the intestine contained no glucuronide. In the cecum and colon a radioactive chromatographic spot was isolated; it contained a fluorescent pigment which appeared to be bound to thyroxine. The R_f of this combination in BuOH:EtOH:NH_4OH and in BuOH:HAc was similar to that of triiodothyronine. The capacity of the intestine to hydrolyze thyroxine glucuronide was measured by in vitro incubation of the I^{131} glucuronide with intestinal contents. Minimal hydrolysis occurred with the contents of the proximal 1/3 of the intestine but progressively greater hydrolysis occurred in material from lower segments. No glucuronide remained after incubation with the contents of the cecum or colon. The major product of hydrolysis was thyroxine.

6 *Physiology*

EFFECT OF DIBENZYLINE ON METABOLIC RESPONSE TO THYROXINE AND EPINEPHRINE. Neena B. Schwartz. Inst. for Psychosomatic and Psychiatric Res. and Train. of Michael Reese Hosp., Chicago.

It has been suggested that the increased metabolic rate (MR) of hyperthyroidism is due to a synergism between thyroxine and epinephrine. Several investigators have attempted to block thyroxine's metabolic effect with Dibenzyline, assuming that it blocks epinephrine's metabolic effect. As we have shown (A.J.P., 191: 573, 1957), when MR is measured at 24°C in anesthetized rats, Dibenzyline lowers MR in hypothyroids and increases MR in hyperthyroids. In the present study MR was measured at 30°C in unanesthetized rats. Under these conditions Dibenzyline lowers MR slightly (a few %) in all rats, this effect being greatest in hyperthyroids. Dibenzyline also lowers MR slightly in euthyroid or hyperthyroid epinephrine-treated rats, but there is no specific "blocking" of epinephrine's metabolic effect. Dibenzyline partially blocks (50%) the hyperglycemic effect of epinephrine. Thyroxine enhances this blocking action. Present and past results suggest that interactions occur between thyroxine and Dibenzyline. However, the Dibenzyline experiments are probably not meaningful in terms of evaluating the role of epinephrine in thyroxine's metabolic actions. (Supported by a research grant, B 1510, from the Natl. Inst. of Neurological Diseases and Blindness, PHS).

7 *Physiology*

THE BASAL METABOLIC RATE OF THE HYPOTHYROID RAT. Pietro O. Bramante (intr. by A.W.Richardson). Dept. of Physiology, St.Louis Univ.School of Med.,St.Louis,Mo.

Oxygen consumption and spontaneous muscular activity of albino rats, with and without anesthesia, were simultaneously and continuously recorded,at thermal neutrality,for some hours, and quantitated by a method previously described (The Physiologist, 2:16,1959. J.Appl.Physiol. 14:1063,1959). The good temporal correlation between the two variables, always present in normal rats, became progressively less evident in a series of experiments performed in the weeks which followed thyroidectomy. Some time after the operation,sudden and unpredictable changes of oxygen consumption (up to 40-50 %) could be detected during the metabolic determination in hypothyroid animals deeply asleep, motionless, and otherwise in 'basal' conditions. Conversely, episodes of more or less intense muscular activity were not invariably accompanied by adequate increments in the oxygen consumption. These observations would indicate: 1.The actual difficulty of establishing true BMR values in the course of chronic studies of hypothyroid animals; 2.The probable futility of performing acute metabolic experiments in the thyroidectomized rat. (Aided by grants from the St.Louis Heart Association and the USPHS.)

Physiology

TIME STUDIES ON THE GOITROGENIC EFFECT OF THYROXINE. E. Schönbaum* and E. A. Sellers. Banting and Best Dept. of Med. Research and Dept. of Pharmacol., Univ. of Toronto, Toronto, Canada.

A diet containing 0.02% propylthiouracil (PTU) and 25μg% L-thyroxine (T_4) caused the formation of much larger goiters than a diet containing PTU only, when fed to rats ad libitum over periods of several months (Science 126, 1342 (1957); Proc. Canad. Fed. Biol. Sci. 2, 59 (1959)). The effect on thyroid size of different dose levels of T_4 (added to the PTU-containing diet) has now been evaluated. 125, 25 and 5μg% T_4 were given over periods of 2, 8, 15, 32 and 64 weeks. 125μg% T_4 reduced the size of the PTU-induced goiters considerably. 25μg% T_4 produced a steady increase in the size of the goiters. Initially the increase in thyroid weights produced by PTU was not altered appreciably by 5μg% T_4, but during the last 32 weeks the T_4 caused much larger goiters and a large variation from animal to animal was observed. The same pattern was found when weights were expressed absolutely or relative to body weight. An exploration of the relationship between type of diet, food intake, and goitrogenesis is in progress. (Supported in part by a grant from the National Cancer Institute of Canada).

9 *Physiology*

THYROXIN STIMULATION OF AMINO ACID INCORPORATION INTO PROTEIN. Louis Sokoloff, Gladys Deibler*, Phyllis Campbell*, Seymour Kaufman*. National Institutes of Health, Bethesda, Md.

It has been reported from this laboratory that L-thyroxin (T_4) pretreatment in vivo or addition in vitro increases the rate of DL-leucine-1-C^{14} incorporation into protein in cell-free rat liver homogenates (Science, 129: 569, 1959). Thyroidectomy has been found to lower this rate. D-Thyroxin is as effective as T_4 in vitro but is relatively inactive when administered in vivo. On the other hand, pretreatment in vivo with 3,5,3'-triiodo-L-thyronine has effects like T_4, but its in vitro action is of lesser magnitude or consistency. The time course of the in vitro T_4 effect at a concentration of $1x10^{-5}$M and an incubation temperature of 37°C indicates a 4-5 minute latent period followed by a 20-minute period of stimulation. After this time the rate of amino acid incorporation in the T_4 flasks returns to the level of the control flasks, but the difference in amounts of amino acid incorporated reached during the stimulation period is maintained. Preincubation at 37°C with thyroxin for 4 minutes eliminates the latent period provided that active oxidative phosphorylation is proceeding during the preincubation; if the substrate for oxidative phosphorylation is absent during the preincubation, the lag period is prolonged rather than eliminated. These results suggest an energy-dependent action of T_4 on protein biosynthesis.

10 *Physiology*

CHANGES IN TISSUE CYTOCHROME C CONCENTRATIONS AFTER THYROIDECTOMY. H. M. Klitgaard and T. M. Hart*. Physiology Department Marquette Univ. School of Medicine, Milwaukee, Wis.

The concentration of cytochrome C was determined in selected rat tissues throughout a period of 28 days after thyroidectomy. The amount of enzyme present was correlated with the previously determined oxygen consumption of the tissue. Cytochrome C remained unchanged during the first 6 days after the operative procedure in heart and diaphragm while O_2 uptake decreased to 75% and 65% of the unoperated control value, respectively. Both tissues showed a sharp decline in enzyme concentration between days 6 and 10 which was followed by a plateau in diaphragm (80%) and a decrease to 55% of control in heart by day 28. Skeletal muscle showed a gradual reduction in the amount of enzyme present after thyroidectomy through day 10 (60%). This was followed by a plateau which was maintained through day 28. Oxygen consumption paralleled the above enzyme changed in skeletal muscle. Kidney and liver exhibited changes similar to those found in heart except for a slight increase in enzyme concentration during the first 6 days after throidectomy. The uptake of O_2 by these tissues correlated poorly with the amount of enzyme present. A decrease in O_2 consumption preceded any reduction in enzyme content in all tissue except skeletal muscle where a close correlation was observed. (Supported by P.H.S. Grant A-957.)

11 *Physiology*

EFFECT OF THYROXINE ON CONTRACTILE EFFICIENCY OF RAT DIAPHRAGM. Robert E. Smith and Wm. J. Whalen*. Dept. Physiol., UCLA Med. Center, Los Angeles, California.

A modified method of Whalen (Am. J. Physiol. 187:640, 1956) was used to measure oxygen cost of isotonic work in rat diaphragm. Bilaterally paired strips were excised from young male Long-Evans rats given 14 daily injections of either saline or l-thyroxine (10 μgm). The respective groups (n = 7) were individually caged and adequately randomized as to weight pairing and sequence of use. Muscles were free-loaded to point of maximal force (~1gm) then after-loaded for duration of 5 hr. test. Alternating with rest, work was induced at rates of 15/min and 30/min on a randomized schedule. Variance within the respective series permitted each to be treated as a single population independently of stimulating frequency. By analysis of variance, regressions of work rate (gm cm/cm^2/hr) on QO_2 (excess over rest) appear highly significant ($P < .001$) and the slope of the thyroxine series significantly lower ($P < .01$) than for controls; respective slopes were 20.45 and 42.76. As QO_2 of rest was constant, the lower efficiency of the thyroxine series must reflect a metabolic alteration incident to work. (Supported by grants from the Natl. Insts. of Health and the Los Angeles County Heart Assoc.)

12 *Biochemistry*

O-METHYL THYROXINE ANALOGS. Kenkichi Tomita and Henry A. Lardy. University of Wisconsin, Madison, Wis.

The methyl ether of thyroxine has about 1/3 the effect of thyroxine on BMR of rats but little or no effect on pituitary production of TSH. To ascertain whether this is a general property of O-methyl derivatives of physiologically active thyronines, other members of this series were synthesized. O-Methyl-L-triiodothyronine (m.p. 210-12°C) is about three times as effective as DL thyroxine in preventing goiter of thiouracil-fed rats. O-Methyl-triiodothyroacetic acid (m.p. 143-5°C) has about 60% the activity of DL thyroxine in this assay and in the BMR test. The O-methyl derivatives of triiodothyronine strongly stimulate adenosinetriphosphatase activity of rat liver mitochondria. These methyl ethers are very effective stimulators of tadpole metamorphosis. Quantitative biological data will be presented. The structures of the new compounds were established by reductive deiodination to yield the methyl ether of the parent thyronine compounds.

13 *Biochemistry*

THYROXINE STIMULATION OF DPNH OXIDATION IN RAT UTERUS. A.T. Bever, C.K. Sokatch and J.H. Anglin. (Intr. by A.C. Kurtz). Univ. of Oklahoma School of Medicine, Oklahoma City, Oklahoma.

Thyroxine as low as $2x10^{-6}$M stimulated DPNH oxidation rates in homogenates of uteri from normal rats but not spayed rats receiving estradiol. Mn^{++} was required. Thyroxine stimulation of DPNH oxidation appears to be related to uterine peroxidase. Inhibitors effective on uterine peroxidase inhibited thyroxine-stimulated DPNH oxidation. Peroxidase values and thyroxine-stimulated DPNH oxidation rates paralleled each other through the normal estrous cycle with maxima in early estrus and minima in diestrus. Peroxidase alone did not insure thyroxine-stimulated DPNH oxidation. Uterine homogenates from estrogenized, spayed rats and the lumenal fluid from uteri of normal rats in early estrus both contained high peroxidase but no thyroxine-stimulated DPNH oxidation activity. Both enzyme sources of peroxidase had 2,4-dichlorophenol-stimulated DPNH oxidase. The pH activity curve for thyroxine stimulation had a maximum at pH 7.4 and was different in shape from the pH curves of dichlorophenol and estradiol stimulation. It is concluded that DPNH oxidation in uterus is stimulated by thyroxine in intact rats but not in spayed, estrogenized rats. The data suggest that mechanisms of stimulation of thyroxine and dichlorophenol are similarly related to peroxidase but are different in other respects. Supported by a grant from the National Cancer Institute.

XUM

118. ENVIRONMENT

14 *Biochemistry*

THE PROTEIN BINDING OF SUBSTANCES RELATED TO THYROXINE. L.K. Christensen (Intr. by K.J. Isselbacher) Mass. General Hospital, Boston, Massachusetts.

By dialyzing serum with added I^{131}-T_4 against the same serum without labeled T_4 an estimate of the non-protein-bound hormone may be obtained. The latter may be considered proportional to the rate at which the I^{131}-T_4 passes the cellophane membrane. Dialysis studies have been performed with different T_4-analogues. The relative concentrations of the free hormones (T_4=100%) are the following: Triac, 230%; Triprop, 176%; Tetrac, 10%; and Tetraprop, 11%. Studies on the erythrocyte uptake of these substances give results which are in qualitative agreement with the dialysis procedure. Addition of non-labeled analogues to serum did not result in an increase in free serum T_4, indicating that they are bound to other sites than T_4. The addition of D-thyroxine resulted in an increase in the free T_4. This might suggest that the physiological activity of D-T_4 is partly due to its L-T_4 displacing effect and not to a direct biological activity of the substance itself. Barbiturate buffer cannot be used in studies on binding of T_4 since it has a T_4 releasing effect.

15 *Biochemistry*

THE EFFECT OF n-BUTYL 4-HYDROXY-3:5-DI-IODOBENZOATE (BHDB) ON THYROID HORMONES. Eunice V. Flook and Jesse L. Bollman.* Mayo Clinic, Rochester, Minnesota.

The antithyroxine activity of BHDB has been attributed to its inhibition of deiodination of thyroxine by Wilkinson, Sprott, Bowden and Maclagan (Biochem. J. 56:215, 1954) and others. It produced a marked decrease in urinary excretion of inorganic iodide after the injection of I^{131}-labeled thyroxine and tri-iodothyronine. We have administered 50 mg. of BHDB orally to rats one day before and on the day of intravenous injection of labeled thyroid hormones to rats. The decreased excretion of iodide in the urine could be accounted for by the increased excretion of I^{131} in the bile. The biliary excretion was tripled in rats given thyroxine or its acetic-acid or propionic-acid derivative. Large amounts of I^{131} from the related tri-iodo compounds also were excreted in the bile, but the increase caused by the drug was smaller, since the normal excretion of tri-iodo compounds is higher than that of tetraiodo compounds. The iodine compounds were excreted in the bile chiefly as conjugates. Glucuronides predominated when the tetraiodo compounds were administered. Glucuronides and sulfates were excreted in almost equal amounts when tri-iodothyronine was given. When BHDB and thyroxine were administered to Gunn rats, there was an increased biliary excretion of I^{131}-compounds which were chiefly sulfates.

16 *Pharmacology*

THE ANESTHETIC PROPERTIES OF THE AZEOTROPIC MIXTURE OF FLUOROMAR (R) AND GENETRON (R) 113. Gordon G. Lu, Johnson S. L. Ling* and John C. Krantz, Jr. Dept. of Pharmacology, Univ. of Maryland Med. Schl., Baltimore.

Fluoromar (R), trifluoroethyl vinyl ether, is a volatile anesthetic (Krantz et al. J. Pharmacol. Exper. Therap. 108: 488, 1953). Attempt was made to reduce its flammability limits, thus explosive hazard, and the expense of manufacturing by the combination with Genetron (R) 113, 1,1,2-trifluoro-2, 2,1-trichloroethane. Both compounds form an azeotropic mixture (AZT) containing 51.2% of Fluoromar (R). In dogs, monkeys and man, AZT elicited a rapid surgical anesthesia with minimal salivation, good muscular relaxation and rapid recovery. AZT retained the same potency and had a comparable Anesthetic Index as Fluoromar (R). In dogs and/or monkeys, deep surgical anesthesia with AZT failed to evoke significant effects on blood pressure, ECG and liver function. Similar EEG patterns were observed in monkeys under AZT and Fluoromar (R). Perfusion of frog heart with AZT solution and the three weeks' follow-up in rats after AZT anesthesia further suggested its non-toxicity. In dogs and monkeys, inducing AZT anesthesia with nitrous oxide-oxygen or cyclopropane-oxygen mixtures was uneventful. AZT possesses a oil/water coefficient of 9515. This is indicative of the high anesthetic potency observed in laboratory animals.

1 *Physiology*

EFFECTS OF STH AND ACTH ON UREA EXCRETION OF RATS ACCLIMATED TO VARIOUS TEMPERATURES AND PRESSURES. Henry B. Hale and Roy B. Mefferd, Jr.* USAF School of Aviation Medicine, Brooks Air Force Base, Texas, and Veterans Administration Hospital, Houston, Texas.

Environmental and hormonal factors are known to influence urea excretion in rats. Both STH and ACTH, when given subcutaneously and in low dosage (0.5 mg/100 gm. B. W.), induced significant reductions in the 24-hour (fasting) urinary urea excretion in rats acclimated to 25° C, and tested at 2-5°, 25°, or 35°. The STH effects exceeded those of ACTH in the tests at 25° and 35° but not at 2-5°. When tested at low barometric pressure (380 mm Hg), the effects of STH and ACTH were qualitatively different, ACTH causing an increase. This was also the case in rats acclimated to low pressure or to heat but not in rats acclimated to cold. When tested under standard conditions (25° C and 750 mm Hg), rats which had been acclimated to cold, heat, or low pressure all responded to ACTH with increases and to STH with decreases in urea excretion. Of greatest importance is the finding that ACTH had STH-like actions under certain conditions.

2 *Physiology*

COLD EXPOSURE DURATION AND AGE EFFECTS ON SELECTED LIVER ENZYMES. John P. Hannon and David A. Vaughan. Arctic Aeromedical Laboratory, APO 731, Seattle, Washington.

The effects of age and of 2 day, 1 month and 4 month cold (4 ± 1^{oC}) exposure on the activities of liver glucokinase, glucose-6-phosphatase, malic dehydrogenase and the rate of pyruvate formation from 3-phosphoglycerate were compared in 2 and 7 month old rats. When calculated on the basis of 100 grams of body weight, increasing age produced a decrease in liver G-6-P-ase and malic dehydrogenase, an increase in glucokinase but no change in the rate of pyruvate formation. Acute (2 day) cold only affected G-6-P-ase, which showed an increase. One month and 4 months cold exposure led to an increased activity in all components except glucokinase. The latter was increased after one month but not after 4 months cold exposure. These experiments indicated that the elevated liver thermogenic capacity was predominantly accomplished by specific activity increases during short term (one month) acclimatization but that this was largely replaced by an increase in the liver to body weight ratio after 4 months acclimatization. A progressive increase in gluconeogenic capacity is also indicated during the course of acclimatization.

3 *Physiology*

COOLING ISOCHRONES. George W. Molnar. US Army Medical Research Laboratory, Fort Knox, Kentucky.

Cooling isochrones are combinations of air temperature and wind velocity which effect in equal times the fall in temperature from an arbitrary initial temperature to an arbitrary endpoint temperature. Cooling isochrones can be calculated by means of formulas appropriate for the object under consideration. Examples for the cooling of an extended finger will be demonstrated. A set of isochrones is valid only for the conditions which are specified: including, geometry and size of the object, its initial and endpoint temperatures, its thermal properties and rate of heat input, etc. Isochrones for the finger are not valid for the trunk, those for the trunk are not valid for a barrel of gasoline, etc.; i. e., there can be no set of isochrones of universal applicability. Isochronous combinations of air temperature and wind velocity do not effect equal rates of heat transfer at a particular surface temperature, and vice-versa. Hence, when the desired information is an index of environments of equivalent cooling rates, the appropriate set of cooling isochrones, and not some scheme based on rates of heat transfer (e. g., the Siple scale of windchill) is indicated.

4 *Physiology*

AUTOMATIC TEMPERATURE COMPENSATION OF ELECTRODES FOR MEASUREMENT OF AVAILABLE TISSUE OXYGEN DURING HYPOTHERMIA; Thomas G. Arnold* and Frank Gollan. Vanderbilt Univ. School of Med., and VA Hosp. Nashville 5, Tenn.

The adoption of a constant temperature coefficient is not permissible. This relationship is not a strictly linear one; it is not reproducible from one membrane of a Clark electrode to another and varies with the rate of temperature change. However, if the temperature curve from 40 to 10° C is divided into two components; if the membrane of each electrode is calibrated and if the temperature does not change faster than 1° C/min., a servomechanism can compensate for the temperature effect. This makes recording independent of temperature changes. The application of this method to measurement of available oxygen in skeletal muscle of dogs during immersion hypothermia showed a rapid fall of 50% to 26° C and a gradual reduction to no measurable oxygen when cardiac arrest occurred at about 18° C. This finding indicates that in spite of normal arterio-venous oxygen difference in large blood vessels severe tissue ischemia develops during immersion hypothermia.

119. ENVIRONMENT–Air Pollution

1 *Immunology*

INACTIVATION OF INFLUENZA VIRUS BY AIR POLLUTANTS. Robert D. Roche* and J.J. Quilligan, Jr., College of Medical Evangelists, Los Angeles, California

C57 Black mice exposed to high concentrations of synthetic smog for 2 months are more resistant to influenza virus infection as determined by LD50 titration and by lesion score, than their corresponding controls, if after inoculation they are again allowed to reside in smog. One of the possible explanations for this phenomenon is destruction of the virus in the infected animals. To study this, various quantities of ozonized gasoline-air mixtures were bubbled through phosphate buffered saline suspensions of egg adapted Lee influenza virus, after which infectivity and hemagglutination were determined. It was found that synthetic smog destroyed egg infectivity though did not affect hemagglutination under the conditions employed. Total oxidant values 1.5 - 3.8 ppm; O_3 0.59 - 1.81 ppm; volumes of smog 5-45 liters.

The effect of O_3 on virus hemagglutination and infectivity was studied by bubbling mixtures of ozone (3.7 - 38.7 ppm) and air through buffered suspensions of virus. Both hemagglutinin and infectivity were destroyed. If, however, virus was incubated with the ozone solution after the gas bubbling was finished, no effect on the hemagglutinin was observed, although the ozone concentrations in solution were substantially the same in both experiments. (Supported by a grant from USPHS.)

120. ENVIRONMENT–Altitude

1 *Pharmacology*

METABOLIC ASPECTS OF POSTMORTEM CHANGES IN HYPOXIA. A.M. Dominguez,* J.R. Halstead,* L.R. Goldbaum, H.I. Chinn, F.W. Lovell,* Armed Forces Institute of Path., Wash. 25, D.C.

Severe hypoxia causes a marked rise in brain lactic acid (LA) which persists after death. This increase in LA has formed the basis of a possible postmortem diagnosis of hypoxia (Am. J. Physiol. 192:577, 1958). In view of the application of this finding to aviation and forensic pathology, factors were explored which might influence postmortem LA content of brain. Since most of the LA presumably arises from LA precursor, i.e. glucose, the relation between blood glucose and postmortem LA response was studied. Adult male rats were exposed to an equivalent altitude of 32,000 feet. After 10 minutes animals were quickly taken to ground level, anesthetized, rapidly bled from abdominal aorta, sacrificed, and LA content of brain determined 4 hours after death. Blood glucose values following hypoxia were 183 mg% ± 4.5 (mean ± S.E.) and in control animals 111 mg% ± 3.6. Brain LA levels in hypoxia and control animals were 179 mg% ± 5.1 and 107 mg% ± 2.3, respectively. Hyperglycemia was induced by glucose infusion, epinephrine, or electric shock, which also caused a rise in postmortem brain LA comparable to levels in hypoxia. Conversely, reducing blood glucose with insulin and then exposing these animals to hypoxia abolished the elevated brain LA. Postmortem levels of lactic acid in the hypoxic brain closely parallels the antemortem concentration of blood glucose.

121. ENVIRONMENT–Hyperthermia

1 *Physiology*

A NEW THEORETICAL HEAT STRESS INDEX. J. Gold (intr. by J. W. Heim.) Aerospace Medical Laboratory, Wright-Patterson Air Force Base, Ohio

A new index, based on heart rate alone, has been devised for the evaluation of physiological strain incurred as a result of heat stress. The index takes into account (a) the beginning and ending heart rates, (b) the same increase in heart rate at different beginning heart rates, and (c) the fact that increase in heart rate cannot exceed a definite value without ensuing physiological deterioration. Results were correlated with body heat storage (q_s) in a series of seventy-five experiments with a resulting overall correlation coefficient of 0.88. Because of this, from heart rate alone it was possible to predict the body heat storage (q_s'), so that the established definitive index was the ratio of predicted over actually measured, body heat storage (q_s'/q_s). Corroborative results were obtained in the Mercury Astronaut Selection Program.

2 *Physiology*

PHYSIOLOGICAL INDEX OF STRAIN AND BODY HEAT STORAGE IN HYPERTHERMIA. John F. Hall, Jr. and J.W. Polte*. Aerospace Medical Laboratory, Wright Air Development Center, Wright-Patterson Air Force Base, Ohio.

In order to determine individual variability of a modified Craig (Chemical Corps Med. Div. Research Report #5, April 1950) index of physiological strain (I_s) and the body heat storage (q_s), and also to define the relationship between these respective parameters, 5 healthy non-acclimatized male subjects were exposed 5 times to each of three heat stress levels: 38°C (100°F), 54°C (130°F), 71°C (160°F) at 10 mm Hg vapor pressure. The index used contains the variables of heart rate, sweat rate and rate of rectal temperature change. These were measured by EKG, nude pre- and post experimental weighings, and with a rectal thermistor, respectively. Body heat storage was calculated from initial and terminal weighted mean skin and rectal temperatures. Subjects were in the sitting state and wore 1 clo of body insulation. Individual and group variation for strain index and body heat storage was determined. The data show an approximately linear relationship between the parameters over the thermal stress range investigated although individual variability was considerable especially at the higher stress levels. A statistically significant correlation between strain index (I_s) and body heat storage (q_s) is shown.

3 *Physiology*

WHOLE BODY EXPOSURE TO TEMPERATURES ABOVE 250°F. Paul Webb, Yellow Springs, Ohio.

Subjects have been exposed without protection to heating in a small chamber in which wall temperatures from 250°F to 500°F (121°-260°C) were combined with air temperatures from 200°F to 450°F (93°-232°C). Two patterns of heating were used - steady state heat, abruptly applied, and a thermal transient termed a slow heat pulse, in which wall temperatures are increased linearly at rates varying from 15°F/min to 100°F/min. Exposures were ended when pain on the bare skin became intolerable. The skin temperature at this intolerable level was measured at several points to be approximately 115°F (46°C). Time to reach tolerance (unbearable pain) was very short for the high levels of heating - 15 seconds for the 500°F wall temperature abruptly applied, and increasingly longer as the rate and amplitude of heating were less. Storage of heat in the body occurred at the lowest levels of heating, these lasting from 4 to 15 minutes. At the lower levels, an increasing storage component and a decreasing pain component suggest a transition to established tolerances for temperatures below 250°F, which terminate from heat storage, not pain. The results have also been analysed to give more data on the value for the thermal inertia of skin.

4 *Physiology*

EFFECTS OF ENVIRONMENTAL TEMPERATURE AND HUMIDITY ON SPREAD OF VIRUS IN THE RESPIRATORY TRACT. A.M. Baetjer, R.H. Lowry* and F.B. Bang. The Johns Hopkins School of Hygiene, Baltimore, Maryland.

Four-day old chicks were kept at 84° and 72°F (the highest and lowest temperatures for survival) with 20 and 90% relative humidity following intranasal inoculation with Newcastle virus. They were killed four to eight days later, and the nares, trachea and lungs assayed for virus. Ciliary activity and tissue temperature were measured in infected and control animals. Histological sections of the respiratory tract were made. When the humidity was high (90%), the spread of virus to the trachea and lungs was greater at a temperature of 72°F than at 84°F. In a warm environment (84°F) the virus spread to the trachea and lungs to a greater extent, the rate of ciliary transfer of a colloidal dye upward in the trachea was slower, and the tissue temperature of the oropharynx and upper trachea was higher when the humidity was 20% than when it was 90%. Microscopic examination indicated that the changes which accompanied the spread of the virus at the low humidity were minimal. Thus the inhalation of warm dry air appeared to inhibit the ciliary mechanism which allowed a greater spread of viral infection in the respiratory tract. (Supported by a grant from the William H. Howell Fund.)

5 *Physiology*

EFFECT OF CLOTHING COLOR ON HEAT LOAD FROM SOLAR RADIATION. J. R. Breckenridge*, R. L. Pratt*, and A. H. Woodcock. Quartermaster Research and Engineering Center, Natick, Mass.

Seated subjects wearing one style of hot-weather Army uniform in four different colors were exposed in the sun and shade during two summers at Yuma, Arizona, to study the effect of fabric color on heat load from absorbed solar radiation. Differences among uniforms were inferred from sweat evaporation rates (obtained by weighings and water balances) converted to equivalent heat dissipations. Subjects in black uniforms evaporated 100 gm/hr more than in white, equivalent to an added load of only 60 kg-cal/hr/man. The differences in green and khaki uniforms were respectively only about half as great, suggesting that changes in color of clothing have a minor effect on heat stress. Absorption coefficients of the fabrics are given, and reasons advanced to explain their poor correlation with solar heat load.

122. ENVIRONMENT–Hypothermia

1 *Physiology*

EXCRETION OF SODIUM AND POTASSIUM IN HYPOTHERMIA. G.S. Kanter. Albany Medical College, Albany, New York.

The purpose of this study was to quantify the renal tubular handling of Na and K at various hypothermic levels. Fourteen dogs were anesthetized with 30 mg/Kg of sodium pentobarbital. After suitable control clearance measurements, the rectal temperature was lowered progressively by ice packing to about 25°C while continous renal clearance studies were carried out. No change in plasma Na was detected but plasma K fell significantly from a control value of 4.1 mEq/L ± 0.09 at 38°C to 3.7 mEq/L ± 0.12 at 27°C. Urine Na concentration fell during exposure to cold while K conc. showed a small increase. In spite of the marked fall in GFR (69.0 ml/min ± 3.1 control to 28.9 ml/min ± 3.4 at 27°C) the final urine flow at 27°C was slightly greater than control. The extraction fractions (E/F) for Na, K, and H_2O increased significantly, reflecting the marked decrease in general tubular reabsorption. The E/F for H_2O increased from 0.49 ± 0.05 at 38°C to 1.21 ± 0.21 at 27°C; the E/F for Na increased from 0.47 ± 0.12 at 38°C to 0.79 ± 0.28 at 27°C, while the E/F for K increased from 18.0 ± 2.6 at 38°C to 39.1 ± 4.6 at 27°C. The variation in effect of hypothermia on the extraction fractions is a reflection of the renal regulations occurring as well as differing reabsorptive mechanisms. (Supported by Contract AF41(657)164, School of Aviation Medicine, Randolph Field, Texas.)

2 *Physiology*

EFFECT OF COLD-STRESS ON ACETATE METABOLISM BY ADIPOSE TISSUE. Judith Patkin, Edith Porter and E.J. Masoro (intr. by A. Canzanelli) Physiology Dept., Tufts Univ. School of Medicine, Boston, Mass.

The effect of cold-stress on acetate metabolism by adipose tissue was investigated. Cold-stress did not affect the ability of the epididymal fat pad to oxidize acetate-1-C^{14} to $C^{14}O_2$. The addition of unlabeled glucose to the incubation medium did not influence the rate of acetate oxidation in the case of adipose tissue obtained from either control or cold-stressed rats. In the absence of unlabeled glucose more fatty acids from acetate-1-C^{14} were synthesized by the adipose tissue from control rats than by that from cold-stressed rats, although very little was synthesized by either. The addition of unlabeled glucose to the incubation medium at the physiologic concentration of 100 mg per cent caused the adipose tissue from both normal and cold-stressed rats to form fatty acids at high rates. It is a striking finding that cold-stress which almost abolishes hepatic lipogenesis does not appreciably alter adipose tissue lipogenesis.

3 *Physiology*

TEMPERATURE SHOCK IN MAMMALIAN SPERMATOZOA. J.K. Sherman. Dept. of Anatomy, Univ. of Arkansas School of Medicine, Little Rock, Ark.

When a population of bovine spermatozoa is cooled rapidly to 0°C, most cells are irreversibly immobilized. Egg yolk protects against such immobilization. Data was presented on the bull, human, dog, rabbit, guinea pig, hamster, rat, and mouse, to show species variation in these aspects of temperature shock. An evaluation of mouse spermatozoa from three levels of the genital tract was made relative to sensitivity to temperature shock, protection by egg yolk, and reproductive capacity of shocked samples. Spermatozoa from the epididymis, vas deferens and ejaculate from brown C57 and white BALB/c mice, 2 to 14 months old, were suspended in .9% saline with and without 20% egg yolk. Samples (0.3 ml) were cooled at an average rate of 0.2°C/sec. Upon rewarming, observations on % motility revealed: 1) Epididymal spermatozoa showed little or no loss, 2) An average reduction of 9% occurred with vas spermatozoa, 3) Ejaculated spermatozoa showed greatest sensitivity to rapid cooling with an average drop of 22%, and 4) Egg yolk did not protect against temperature shock. Results of artificial insemination of shocked populations proved that some spermatozoa retain their fertilizing capacity and effect production of normal progeny. (Supported by a grant from Division of Research Grants, Nat. Inst. Health.)

4 *Physiology*

METABOLIC EFFECTS OF UREA ADMINISTRATION ON ACUTE HYPOTHERMIA IN RATS. John R. Beaton* and John Hunter. Defence Research Medical Laboratories, Toronto, Canada.

As part of a long-term investigation of hypothermia, it was found that intraperitoneal injection of urea (120 mg/100 g body weight) is effective in permitting successful resuscitation and survival of rats cooled to rectal temperatures of 0-3°C. In the absence of therapy, cooled fasted rats show a significant decrease in blood glucose and pH and a significant increase in blood lactic acid, packed cell volume and inorganic phosphate with no alteration in blood pyruvic acid, urea, amino nitrogen or acid-soluble phosphate. Treatment with urea prior to cooling prevented the significant decrease in blood glucose and increase in blood lactic acid. A study of the effects or urea administration in non-cooled rats has been carried out. In non-cooled rats a single injection of urea (120 mg/100 g body weight) produced a significant elevation in blood glucose levels. Preliminary evidence suggests that prolonged daily administration of urea to non-cooled rats results in glycosuria evident after a few days of treatment.

5 *Physiology*

EEG AND HEART RATES OF HYPOTHERMIC CATS. J. A. Lipp*, J. R. Knott*, and G. E. Folk, Jr. Dept. of Physiology and The EEG Laboratory, Univ. of Iowa, Iowa City.

The metabolism of the brain has been reported to drop 6.7%/degree drop in temperature during hypothermia. The present paper submits data on the cerebral activity of hypothermic cats. Dural thermocouples and silver electrodes were implanted using acrylic screws. Subcutaneous tantalum rings were implanted in the sides of the cats for recording the EKG. Hypothermia was obtained by adding crushed ice, after a calm control reading was taken. The data fall into two categories: (A) with the first 7° C drop in body temperature (heart rate: 35% drop), the type A cats exhibited bursts of high amplitude 5-7/sec waves; with the second 7° drop (heart rate: 70% drop), the EEG records became very low in amplitude; (B) with the first 7° drop in body temperature (heart rate: 34% drop), the cats did not exhibit any slow, high voltage bursts; with the second 7° drop, the results were the same as type A. The sequential physiological changes could be explained in terms of an early selective action of hypothermia on the midbrain reticular area. (Financed in part by National Science Foundation and U.S.P.H. M-675.)

6 *Physiology*

EFFECTS OF COLD-ACCLIMATION ON HAMSTER CELLULAR ENZYME ACTIVITY. R.R.J. Chaffee,* R.T. Clark, J. Lowe* and W.L. Bartlett.* School of Aviation Medicine, USAF, Brooks AFB, Tex., U. of Redlands, Redlands, Cal., U. of C., Berkeley, Cal.

In the hamster, Mesocricetas auratus, weeks or months of exposure to cold are prerequisite to hibernation. Studies of the effects of this period on activity of cellular enzymes were conducted in order to gain information concerning preparation for hibernation in these animals. Activity of succinoxidase (SO), succinic dehydrogenase (SDH), and glutamic-oxalacetic transaminase (GOT) were assayed in homogenates of kidney from hamsters acclimated at 5 ± 2°C. for 3-4 weeks, and controls at 28 ± 4°C. Also assayed was β-hydroxy butyric acid oxidase (BAO) activity of liver mitochondria. The mg. of N/mg. wet wt. of kidneys was determined. Acclimated hamster SO, SDH and BAO were significantly higher than control values. GOT was not affected. Acclimated kidney wt. was 46% greater than the control, but the mg. N/gm. wt. was unchanged, indicating increased wt. represented protein deposition. Since some hamsters (not used for these experiments) hibernated, experimental acclimation conditions apparently simulated natural prehibernation environmental conditions. Possibly a series of intracellular enzymatic adjustments must take place before hibernation can occur in the hamster and this could in part account for the necessity of the cold acclimation period.

7 *Physiology*

EFFECT OF DNP ON CONTROL OF ANESTHETIC HYPOTHERMIA. Shannon C. Allen and Edward L. McGovern *. Dept. Physiology and Pharmacology, Chicago College of Osteopathy, Chicago, Ill.

Earlier work on the influence of cold on the calorigenic action of DNP and certain observations in our teaching laboratories led to the attempt to test the possibility that this drug might be used as a control measure in the use of hypothermia as an anesthetic technique in surgery. ECG's, core temperatures and respiratory rates were recorded from unanesthetized rabbits during reduction of body temperature to ca 30°C with an ice-water bath and rewarming to control levels. This is being done before and after the injection of DNP so that each animal acts as its own control. The study is being expanded to include other species and the data will be discussed on that basis.

8 *Physiology*

RELATIONSHIP OF HIBERNATION TO CONTRACTILITY AND IRRITABILITY OF DIAPHRAGM MUSCLE AS AFFECTED BY TEMPERATURE. Frank E.South Dept. of Physiology, U. of Ill. Coll. of Med., Chicago.

Diaphragm preparations were incubated at various temperatures, ranging from 5° to 38° C. The muscles were obtained from control rats, control hamsters and from hamsters hibernating at 5°C. The effect of temperature on relative thresholds, rates of contraction and relaxation, and tension production of single twitches and tetanic contractions were examined in the three groups of animals. Stimuli were delivered both directly and via the phrenic nerve. The apparent energies of activation for rates of tetanic contraction and relaxation from both twitch and tetanus were highest for the rat muscles and lowest for those of the hamsters. The opposite situation obtained for rates of twitch contraction, except at the lower temperatures. Thresholds to both indirect and direct stimulation (with curare) was found to be much lower for hamsters than the rats - particularly at temperatures of 17°and below. Tension production was greatest at all temperatures and least temperature sensitive, in the lower range, for the hamsters. Most dramatic was the fact that myoneural transmission failed in the rat preparations at 10° and in those of the control hamsters at 5°. Those of hibernating hamsters responded readily at all temperatures. (Supported by the ONR, Nonr-1459-05, and the NIH, RG-5305.)

9 *Physiology*

THE REDUCTION IN HEAT STRESS RESULTING FROM REST PERIODS SPENT IN A COOL ENVIRONMENT. Gordon J. Stopps*, Lucien Brouha, Mary E. Maxfield and Paul E. Smith, Jr.* Haskell Laboratory for Toxicology and Industrial Medicine, Newark, Delaware.

The work consisted of walking on a treadmill at 3.2 mph and 5% grade in an environment controlled at 105°F and 65% RH. First the subjects performed four 15-minute walks separated by 20-minute rest periods in the hot environment. In a second series each walk was increased to 25 minutes, the recovery period was reduced to 10 minutes but took place in a cool environment (76°F, 50% RH). In both cases the total experiment lasted 140 minutes. Physiological data will be presented showing that the cardiovascular stress is increased only 2% while the work performed is 67% greater. Furthermore, rest in the cool environment enabled an essentially steady state to be maintained for both heart rate and body temperature, whereas continuous heat exposure caused a progressive rise in both.

10 *Pharmacology*

EFFECTS OF HYPERCAPNIA AND HYPOXIA DURING HYPOTHERMIA. Mearl A. Kilmore * and Harold F. Chase Jefferson Medical College, Philadelphia, Pennsylvania.

It has already been reported that the response to respiratory obstruction during hypothermia is less than during normothermia. It is possible that this decreased response may be due to either an increased tolerance to hypercapnia or to hypoxia. In dogs under thiopental, nitrous oxide, oxygen anesthesia 5% CO_2 was administered to one group and 15% O_2 to another group. In both the hypercapnic and hypoxic groups the response of arterial, inferior vena caval, external jugular, cisternal and endotracheal pressures were essentially the same under normothermia and hypothermia for each group. In the hypercapnic group, both minute volume and respiratory rate are diminished in response to CO_2 during hypothermia as compared to the response to CO_2 during normothermia. The ventilatory response to the hypoxic stimulus in hypothermia is less uniform. (Supported by grant # H-4033 from the United States Public Health Service).

124. GASTROINTESTINAL TRACT

1 **Physiology**

Esophageal Pressure Waves While Swallowing Liquids In The Vertical and Horizontal Position. A. R. Vela* and J. P. Quigley, Div. of Physiology, Univ. of Tennessee, Memphis

Intra-esophageal pressure changes were recorded in three normal humans by a modification of the method of Quigley and Brody (Am. J. Med. 8:1, 1952). In the vertical position, the peristaltic wave following a single swallow of 20-30 ml of water began at the pharyngoesophageal junction, traversed only the striated muscle in the upper 1/3 of the esophagus (18 to 24 cm from incisors) and terminated at the junction between the lower cervical and upper thoracic esophageal segments. In the vertical position there was no pressure change (wave) at the junction area resulting from the swallowing. A new peristaltic wave originated at the junction between the lower cervical and upper thoracic esophageal segments and traversed the smooth muscle in the remaining 2/3 of the esophagus without interruption. In the supine position, the peristaltic wave initiated by a single swallow of 20-25 ml of water swept uninterrupted through the junction between striated and smooth muscle. Fluid swallowed by a subject in the vertical position passes through the portion of the esophagus where striated muscle is replaced by smooth muscle by gravity and does not evoke the pressure wave which is required to propel fluid through this region when the subject is in the horizontal position. (Supported by PHS grant A 1764)

2 **Physiology**

METHOD FOR MEASURING THE GAS EXCHANGES AND CLEARANCE OF CO_2 PER CENTIMETER OF INTESTINAL MUCOSA PER MINUTE. Alfred W. Brody, J. William Knox,* Reginald S.Carvalho,* James J.Navin,* and Elmer E. Kobold.* Chair of Cardiovascular Research, Dept. Med., Univ. Creighton Med. School, Omaha, Nebr. Supported in part by Nebraska Heart Assn.; in part by the National Foundation.

In a series of anaesthetized dogs a segment of small intestine was exposed and intubated. Pure oxygen was injected at a slow steady rate between 2 occluding balloons and allowed to exchange with intestinal mucosa. The resulting gas mixture was collected through a flow meter and gas chromatography analysis system into a spirometer, thus measuring flow rate and CO_2 content. A blood CO_2 tension-content curve was prepared in each dog. Arterial analysis or continuous gas chromatography of end-expiratory (alveolar) samples provided a measure of arterial CO_2 tension. Clearance of mucosal blood of CO_2 was calculated in cc per cm of length. This provides a lower limit of mucosal blood flow. Comparison with nitrogen (an inert gas) clearance was made by similar techniques.

3 **Physiology**

CHLORIDE, SODIUM AND INULIN SPACES OF RAT DIGESTIVE GLANDS. L. H. Schneyer and C. A. Schneyer. Depts. of Physiology and Dentistry, Univ. of Alabama Med. Ctr., Birmingham, Ala.

Chloride, sodium and inulin spaces have been determined for the pancreas and parotid and submaxillary glands of the rat. Inulin space differs in the three glands being greatest in parotid and least in pancreas. Chloride and sodium spaces exceed inulin space in all three tissues, thus suggesting the presence of chloride and sodium intracellularly. (Supported by a grant from Natl. Insts. of Health.)

4 **Physiology**

EFFECT OF MORPHINE ON ELECTRIC AND MOTOR ACTIVITY OF THE DUODENUM. Paul Bass,* Charles F. Code and Edward H. Lambert. Mayo Clinic and Mayo Foundation, Rochester, Minnesota.

This investigation was undertaken to determine the quantitative relationships between the slow basic electric rhythm, the irregular bursts of spike potentials and the motor action of the duodenum of the dog during fasting and after the administration of morphine. Observations were made on four trained, unanesthetized dogs prepared with a Biebl loop containing the distal third of the duodenum. Needle electrodes arranged longitudinally and radially in the wall of the duodenum detected electric activity; an open-tip polyethylene tube in the lumen registered pressure. The slow basic electric rhythm was recorded simultaneously from the radially arranged electrodes, and in sequence from electrodes placed longitudinally, suggesting conduction distally at 19 cm. per second. In control periods spike potentials, although irregular in occurrence, correlated well with motor contractions. Morphine induced rhythmic motor activity in the bowel. The rate of the contractions then corresponded to the basic electric rhythm, and each contraction was preceded by a burst of spike potentials. Morphine increased the duration of the bursts of spike potentials and the number and amplitude of the spikes in each burst. The mean amplitude of the basic electric rhythm was reduced, but the duration and frequency of the cycles were unaffected. In control and morphine periods spike potentials only occurred in a specific segment of the cycle. (Supported by a grant from Public Health Service, #A2015.)

5 **Physiology**

LEUCINE INCORPORATION INTO CELL TYPES OF COLONIC MUCOSA. Martin Lipkin and Thomas P. Almy. Gastrointestinal Lab., Second (Cornell) Medical Division, Bellevue Hospital, New York 16, N.Y.

Incorporation of C^{14} and tritium labeled leucine into proteins of colonic mucosa of C 57 Brown mice has been measured with chemical and microautoradiographic techniques. Specific activity measurements of incorporation into whole mucosal protein residue have revealed rapid incorporation and removal of label. The findings have been correlated with microautoradiographic measurements of incorporation into cell types of colonic mucosa. Leucine has been noted to incorporate in high concentration into cytoplasm of epithelial cells lining the base of the crypts and intestinal lumen. Epithelial cells between crypt base and intestinal lumen incorporate leucine in lower concentrations. Goblet cells and connective tissue also incorporate less leucine than the crypt and surface epithelial cells. The patterns of incorporation noted at early and later intervals following injections of leucine are being compared to evaluate changes occurring during migration of epithelial cells.

6 **Pathology**

HISTOLOGIC METHOD OF DEMONSTRATING INTESTINAL FAT ABSORPTION IN MAN. Cherill Parmentier, Patricia Phelps and Cyrus E. Rubin (intr. by Robert Priest) Univ. of Wash., School of Med., Seattle, Wash.

With a new hydraulic instrument any number of mucosal biopsies may be taken safely and painlessly from any level of the gastrointestinal tract and delivered exteriorly within seconds of excision. This instrument passes orally to the terminal ileum. The test begins following a day's fat-free diet and an overnight fast. After a control ileal biopsy, 60 cc of Ahren's corn oil formula is instilled; the same site is rebiopsied at 3 and 10 minute intervals under x-ray control. This procedure is repeated at 7 ascending levels in the ileum, jejunum and duodenum. After careful orientation on paper each specimen is fixed in Bouin's or Baker's formalin and blocked in equal parts of Carbowax 1,000 and 4,000 which can be thinly sectioned at room temperature. The sections are floated with Wade's Tergitol 7 and affixed to the slides with gelatin, glycerol, phenol solution. Multiple Tergitol 7 rinses of the near-dry slides removes obscuring background. Staining by Sudan Black-Nuclear Fast Red or Oil Red O-Ehrlich's Hematoxylin reveals exquisite details of fat absorption, i.e. individual fat particles between discrete microvilli. Parallel electron microscopy has proved feasible. (Supported by USPHS grant Path C-4320.)

Pathology

7

SERUM ALBUMIN (ALB) IN CANINE GASTRIC ACETYLCHOLINE-MUCUS (ACh-Mu) AND WHOLE STOMACH ASPIRATES. F. Hollander and M.I. Horowitz*. GE Res Lab., Mt. Sinai Hosp., N.Y.

The multi-component hypothesis to explain the variable composition of gastric juice postulates that interstitial fluid transudate may be one of these components. Electrophoresis of ACh-Mu from Heidenhain pouches showed that the leading anodal peak (LAP), containing 30-50% of total protein, had the same mobility as canine Alb. After I.V. injection of I^{131}-Alb, practically all protein-bound I^{131} in the ACh-Mu fell under LAP; the non-dialyzed secretion contained considerable amounts of non-protein-bound I^{131} also. When I^{131}-Alb was administered to unoperated dogs, aspirated anacid secretions contained protein-bound I^{131}. Acid aspirates also contained considerable I^{131}, of which only small amounts were protein-bound. Absence of LAP-protein from ACh-Mu following incubation at pH 1.2 suggests loss of I^{131}-Alb by peptic degradation. Hence, Alb (and therefore interstitial fluid) may be a normal constituent of gastric secretion which is not found in ordinary acid specimens because of proteolysis. These results are important in relation to the clinical concept of exudative enteropathy. [Grant #C-288, NCI, USPHS.]

Pathology

8

In Vitro and In Vivo Radioautography of Gastrointestinal Tract Neoplasms. D.B. Shahon,* N.E. Ackerman,* O.H. Wangensteen Dept. of Surgery, Univ. of Minn., Minneapolis, Minnesota

Malignant neoplastic tissues have demonstrated an ability to incorporate larger amounts of radioactive phosphorus than do normal tissues. Following IV administration of 500 mc. of P32 12 to 24 hours prior to surgery, the excised specimen is cleared of serosal fat and mesenteric attachments and washed, and mounted to obviate mucosal folds and ridges. The specimen placed face down on a single, thin sheet of Saran Wrap is taped to a hard board cassette forming an envelope, permitting insertion of an x-ray film opposite the mucosal surface, and separated from it by the thin Saran Wrap sheet to prevent digestion of the photographic emulsion on the x-ray film. The specimen and x-ray film are inserted into a black-lined cardboard box and placed in a refrigerator for 12 to 24 hours. These studies indicate very definitely increased uptake of P32 by malignant tumors. Benign gastric ulcers have a lower P32 uptake than normal gastric mucosa. Radioautography has proved helpful to the pathologist in indicating where sections should be made to find an occult neoplasm. These studies suggested that in vitro gastric radioautography might be useful in the preoperative detection of gastric cancers. The technique of achieving such in vivo radioautographs of the stomach will be described. (Support-Ca. Tr. Grt. and Mal. Dis. Research Fund)

Pathology

9

INTESTINAL CARCINOMA IN RATS FED N-2,7-FLUORENYLENEBISACETAMIDE. Harold L. Stewart, Katharine C. Snell*, Harold P. Morris, and Billie P. Wagner*. National Cancer Institute, Bethesda, Md.

One or more primary carcinomas of the jejunum or ileum were found in 14 of 18 Buffalo male, in 9 of 14 Buffalo female, in 11 of 13 AxC male, and in 9 of 14 AxC female rats fed a basal diet containing 0.025% N-2,7-fluorenylenebisacetamide. One Buffalo female had a total of 7 carcinomas, the largest number in any single animal. The majority of the tumors were located in the lower jejunum and upper ileum, frequently at the site of a lymph follicle. Three tumors in Buffalo male rats metastasized to the lung, spleen, and peritoneum. One carcinoma of the colon was found in a Buffalo female rat, and one carcinoma of the duodenum in an AxC female rat. Atrophy and atypicality of Brunner's glands were present in the duodenum of all rats examined, but atrophy of the intestinal mucosa was, in general, not a prominent feature.

Pathology

10

EFFECTS OF CHLORPROMAZINE ON A TRANSPLANTABLE MOUSE MASTOCYTOMA. L. S. Gottlieb*, M. Hazel*, S. Broitman* and N. Zamcheck. Mallory Institute of Pathology and Tufts, Boston Univ. and Harvard Schools of Medicine, Boston, Mass.

Chlorpromazine has been reported to alleviate some clinical symptoms of the human carcinoid syndrome and to have a regressive effect on rat hepatomas. Accordingly, the effects of CPZ on a transplantable serotonin-producing mast cell tumor in the mouse were studied. Tumor suspensions were injected subcutaneously into DBA/2 and AKD/2 mice. From the 7th to the 15th day a daily 1 cc. solution of CPZ (25 mg.) was injected intraperitoneally. Controls were injected I.P. with 1 cc. of 0.85% NaCl. The animals were sacrificed 15 days after injection and were autopsied immediately. Tumor, spleen and liver were removed, weighed and fixed in 10% formalin. The amount of histologic metastases to liver and spleen stained with H and E and 0.5% toluidine blue were graded on a 0-6+ scale. The CPZ treated animals had significant reductions in tumor weight (31%), splenic weight (46%) and splenic mast cell metastases. There were no effects on hepatic weight, hepatic metastases or tumor morphology. Urinary 5-HIAA excretion, as measured by paper chromatography, averaged 30.8 gamma/day/animal for the last 5 days as compared with 70.5 gamma in control animals.

Pathology

11

THE EFFECT OF DIETARY DEPRIVATION & ALCOHOL ON THE ABSORPTION OF XYLOSE FROM THE RAT SMALL INTESTINE. M.D. Small*, S.N. Gershoff*, S.A. Broitman*, P.L. Colon*, and N. Zamcheck. From Boston City Hospital, Dept. of Nutrition, Harvard Sch. of Public Health and Boston Univ. Med. School.

Abnormalities of urinary xylose excretion have been noted in Skid-row alcoholics following acute drinking episodes. The effects of deficient diets, alcohol & congeners on the intestinal absorption of xylose in weanling rats were studied by Small's modification of the Cori technique. 1) Three week study: there was a reduction in xylose absorption in animals receiving a normal diet with 20% alcohol, and a diet deficient in protein, fat, water-soluble vitamins, lacking in vitamins A & D with and without alcohol. 2) 4 month Study: there was a decrease in xylose absorption in animals fed a 10% protein diet with alcohol as compared to rats on a 20% or 10% protein diet without alcohol. A slight reduction in absorption was noted in rats on a 20% protein diet with alcohol. 3) 1 year Study: there was a decrease in xylose absorption in rats fed a 10% protein diet with alcohol, with or without congeners, as compared to rats receiving a similar diet with water. 4) the youngest control animals absorbed more xylose than did the other controls. Alcohol and/or dietary deprivation can produce malabsorption in rats.

Pathology

12

INTESTINAL ABSORPTION AND URINARY EXCRETION OF XYLOSE IN RATS FED REDUCED PROTEIN AND THYROXINE OR ALCOHOL. S.A. Broitman,* M.D. Small,* J.J. Vitale and N. Zamcheck. Boston City Hospital and Dep't. of Nutrition, Harvard School of Public Health and B.U. School of Med., Boston, Mass.

The effect of thyroxine, 20% alcohol and reduced dietary protein on urinary output and intestinal absorption of d-xylose was studied in the rat. Five and 24 hr urinary outputs were measured after intragastric instillation of a 2ml solution of 120 mgm of xylose. Intestinal absorption was determined by measuring the residual xylose in the gut lumen 1 hr after intraduodenal instillation of 500mgm of xylose in 2ml of water. Animals fed 0.1 mgm% thyroxine exhibited 5 hr urinary xylose outputs approx. 70% greater than control animals. However, the intestinal absorption of xylose in thyroxine treated animals did not differ from control animals. No differences were observed in either urinary excretion or intestinal absorption of xylose between groups of rats fed 20% protein and alcohol and those fed water. Reducing protein alone did not alter excretion or absorption of xylose. But the addition of alcohol caused a decrease in both 5 hr urinary xylose output and intestinal xylose absorption in animals fed a 10% protein diet. Thus although the 5 hr urinary output did not always reflect the intestinal absorption of xylose, a reduced output was indicative of xylose malabsorption in rats.

XUM

125. GASTROINTESTINAL TRACT—Absorption

1 *Physiology*

IN VITRO STUDIES OF INTESTINAL ABSORPTION IN THE TURTLE, Chrysemys picta. Sister Alice Marie Fox, BVM* and X. J. Musacchia. St. Louis Univ., St. Louis, Mo.

Glucose absorption by isolated pieces of turtle intestine was studied in vitro. Comparisons were made of absorption by the intestines dissected from turtles at room temperature (28°C) and those under induced cold torpor (2-4°C). Some of the results indicate that at 10°C or higher, there is active transport of glucose from the fluid at the mucosal surface to the fluid at the serosal surface. Terminal concentration gradients were highest (11.4) for the smallest glucose concentration used (1.1 mg.%), intermediate (6.6 and 10.3) for 2.25 and 4.5 mg.%, respectively, and lowest (3.4 and 1.6) for the two most concentrated solutions (9.0 and 18.0 mg.%). Transport at 28°C (in uM glucose/gram wet weight/30 min.) showed an increase of 0.24 umoles to 0.47 umoles with increasing concentrations in a range from 1.1 mg.% to 9.0 mg.%, and little or no transport with 18 mg.% glucose. The intestine of 4°C cold torpid animals released glucose into the serosal fluid, possibly by diffusion alone. Temperature effects on transport were investigated and maximum absorption appeared to take place at 28-30°C, and minimum at 4°C. Concentration gradients established by portions of the lower small intestine were significantly lower than those observed from duodenal portions.

2 *Physiology*

FAT EXCRETION IN DOGS LACKING BOTH BILE AND PANCREATIC JUICE. Bennett J. Cohen (intr. by R. R. Sonnenschein.) Dept. of Physiol., UCLA School of Medicine, Los Angeles.

Long chain lipids are absorbed via lymphatic channels (Biochem. J. 58:600, 1954). However, rats deprived both of bile and pancreatic juice absorbed little or no corn oil or its constituent fatty acids (A.M.A. Arch. Surg. 69:247, 1954). The implication that little or no fat absorption occurs in combined bile-pancreatic deficiency was tested in 7 day balance experiments. Dogs deprived of pancreatic juice (4 dogs), bile (3 dogs), or both (6 dogs), were fed a basal meal (10 g. lipid), and the basal diet plus 25 g. or 50 g. of triolein, and 10 g. or 20 g. of oleic acid. 5 day fecal collections were analyzed for total petroleum ether soluble material. In pancreatic deficiency triolein excretion was 48.8% of the intake; oleic acid was completely absorbed. In bile deficiency the triolein defect was 62%; 10 g. of oleic acid, but not 20 g., was absorbed completely. Triolein and oleic acid excretions were 57.7% and 62.9% respectively in the combined defect. Thus, assimilative defects are not additive, and substantial fat absorption occurs, in dogs lacking both bile and pancreatic juice. (Supported by grant A1941 from N.I.A.M.D., Public Health Service.)

3 *Physiology*

ON THE "ABSORPTION PRESSURE" OF RAT INTESTINE DURING WATER ABSORPTION IN VITRO. J. S. Lee (intr. by N. Lifson.) Dept. of Physiology, Univ. of Minn. Minneapolis, Minnesota.

In the isolated intestine preparation of Smyth and Taylor, with the mucosa lavaged with physiological salt solution, fluid flowed out from cannulae in the mesenteric vein and lymphatic duct. The maximum pressures developed in these vessels with outflow occluded will be called "venous" or "lymphatic absorption pressure" (VAP or LAP), with the zero base at the average level of the suspended loop. The absorption pressure was affected by the hydrostatic pressure (HP) of the lavage fluid, but it was not a simple reflection of HP. The absorption pressure was positively correlated with water absorption by the mucosa. In the absence of such absorption (anoxia, no glucose in lavage fluid, addition of NaCN (0.2%) or phlorhizin (0.1%) the VAP was zero even when HP was as high as 68 mm Hg. In 6 out of 20 determinations on jejunum-ileum loops, the VAP ranged from 18 to 25 mm Hg; in the remained cases together with 16 duodenum-jejunum loops the VAP ranged from 5 to 17 mmHg. Simultaneous determinations of pressures showed that LAP was always significantly higher than VAP. The HP ranged from 20 to 25 mmHg in this series of experiments.

4 *Physiology*

EFFECT OF OLEIC ACID ON THE ENDOGENOUS FECAL EXCRETION OF STEROL IN THE DOG. R. Wojciech (intr. by W.H.Bachrach), Univ. Illinois College of Med., Chicago, Ill.

A synthetic diet containing no sterol and no fat was fed to 5 dogs. The diet contained 30% casein and was fed at a level of 75 calories/kg/day, all being consumed. The feces were collected daily. The diet was fed for 11 days, the feces being discarded the first 4 days and collected the following 7. The average weight of the dogs was 13.5 kilos. The average amount of endogenous digitonin precipitable sterol excreted was 138 mg/day, the range being 102 to 189 mg. When oleic acid (sterol free) was added to the diet at a level of 6% by weight, the caloric intake being maintained the same by the reduction of dextrose, the average sterol excretion was 144 mg/day, the range being 102 to 213 mg. Data will be presented showing that the percentage absorption of cholesterol is less when fed alone just before the meal than when it is thoroughly mixed with the meal. This is important because some have fed sterols as pellets.

5 *Physiology*

RELATION OF CHEMICAL STRUCTURE OF CHOLANIC ACIDS TO THE INTESTINAL ABSORPTION OF CHOLESTEROL. R. Suzuki (intr. by R.C. Ingraham), Univ. Illinois College of Med., Chicago, Ill.

Chronic bile fistula rats were used. The cholesterol (50 mg of cholesterol, a tracer of C14-4-cholesterol) was dissolved in ether and the solution was added to the synthetic-sterol-fat-free daily diet (10 gm). The ether was evaporated and the Na salt of the bile acid (300 mg to 370 mg, or .55 to .77mM) was added to the mixture. After mixing, 0.33 cc oleic acid was added and mixed, enough water being added to make a thick paste. This paste was divided into six aliquots, one of which was fed every 2 hours for 12 hours. The radioactivity in the bile and feces was determined. The following is the average percentage cholesterol absorption for 5 rats: taurocholate, 44.7; glycocholate, 40.7; cholate, 21.4; desoxycholate, 3.5; apocholate, 1.9; hyodesoxycholate, 1.0; lithocholate, 1.0; dehydrocholate, 1.1; taurocholate without oleic acid, 16.7. Cholic acid and two of its conjugates was the only cholanic acid used which definitely rendered possible the absorption of cholesterol. The chemical mechanism involved will be discussed.

6 *Physiology*

NEW THIRY FISTULA METHOD USED TO STUDY ABSORPTION OF THIAMINE BY THE JEJUNUM AND ILEUM. Alberto Carvalho da Silva (intr. by M.J.Schiffrin), Univ. Illinois College of Med., Chicago, Ill.

I have prepared with Dr. A.C.Ivy a Thiry fistula of the jejunum and ileum which avoids leaks and renders complete recovery of introduced fluids possible and which may be used indefinitely. To date six dogs have been studied, 3 with a jejunal fistula and 3 with an ileal fistula. When 20 ml of normal saline containing 10 mg of thiamine hydrochloride was introduced into the fistualized loop and 30 seconds later the loop was drained and irrigated with saline, the average recovery of the thiamine in 15 tests was 100.2%, the range being from 70 to 124%. When 20 ml of a saline solution containing 10 mg of thiamine hydrochloride was introduced into the fistualized loop of the jejunum for 1 hour, an average of 32% was absorbed. In the case of the fistualized loop of ileum, an average of 29% was absorbed. Thus, the rate of absorption of thiamine is approximately the same from the jejunum and ileum under the conditions of the tests. The contents of the loop, when incubated for 12 hr. with thiamine, did not destroy thiamine. The absorption of glucose from the loops was normal, and Vischer et al was confirmed in regard to a faster rate of absorption of chloride from the ileum than the jejunum

Biochemistry

1

INFLUENCE OF PYRIDOXINE, PYRIDOXAL, PYRIDOXAL PHOSPHATE, DEOXYPYRIDOXINE AND 2,4-DINITROPHENOL ON L-TYROSINE ABSORPTION. F. A. Jacobs, R. C. Flaa and W. F. Belk (intr. by H. J. Fromm.) Dept. of Biochem., Univ. of N. Dak. School of Med., Grand Forks, N. Dak.

The influence of two antagonists and the B_6-vitamins on the absorption of L-tyrosine in the upper small intestine of the rat has been investigated by an in situ perfusion technique essentially as previously described (Jacobs and Hillman, J. Biol. Chem. 232:445, 1958). Intestinal segments were perfused for 1-hr periods with 0.002 M L-tyrosine in 0.85% NaCl. 2,4-Dinitrophenol (DNP) (10.0 mg/kg) and deoxypyridoxine.HCl (0.5 mg) were injected 1 hr before perfusion. The vitamin factors were injected before or after the antagonist at a dose level equimolar to the deoxypyridoxine. Pyridoxine.HCl, pyridoxal.HCl and pyridoxal phosphate neither stimulated nor depressed tyrosine absorption when administered without antagonist. Deoxypyridoxine-inhibited absorption was prevented by pyridoxal. DNP-inhibited absorption was alleviated by pyridoxal phosphate but not by pyridoxine nor by pyridoxal, however these latter B_6-vitamins prevented the onset of DNP inhibition. Measurements were made in respect to absorption rates as well as the net load absorbed. (Supported in part by NIH Grant No. A-2023).

Pharmacology

8

STUDIES OF THE METABOLISM OF RHODAMINE B (D&C RED 19). Junius M. Webb (introduced by O. Garth Fitzhugh). Div. of Pharmacology, Food & Drug Admin., U. S. Dept. of Health, Education and Welfare, Washington, D. C.

Following ingestion of the dye by rats, three dye metabolites were found in the urine and they were also present in the feces. The metabolites were detected in U V light by their fluorescence and Rf characteristics on paper chromatograms. Evidence indicates the dye is metabolized by deethylation of diethylamine groups and that the basic phthalein structure remains intact. The study has been extended to (1) metabolic pattern in other species; (2) site or sites of metabolism in rats. In the first instance preliminary studies have shown the same pattern in dogs and rabbits as in rats. In the second instance examination of the bile following cannulation of the bile duct and intravenous injection of the dye, and examination of minced intestines and intestinal contents incubated with the dye, revealed none of the three metabolites. This does not eliminate the liver as metabolic site, but suggests that the dye must recirculate.

Pharmacology

9

THE METABOLISM OF FD&C RED NO.1. I. THE FATE OF 2,4-META-XYLIDINE IN RATS. H. V. Lindstrom (intr. by E. P. Laug.) Div. of Pharmacology, Food and Drug Admin., U. S. Dept. of Health, Education and Welfare, Washington, D. C.

The rupture of the N=N linkage in FD&C azo dyes by living systems results in the formation of naphthylamines and anilines. In rats fed FD&C Red No.1, the naphthylamine segment (1-amino, 2-naphthol 3,6-disulfonic acid salt) is water soluble and appears to be eliminated unchanged; the aniline segment is a mixture of water-insoluble amines (pseudocumidine, 2,4-meta-xylidine, 2,6-meta-xylidine, 2,5-para-xylidine and others) which are altered structurally by the animal. Much toxicological work with "xylidine" has been performed with mixtures of xylidine isomers; our study considers these isomers separately to elucidate differences in their metabolic behavior. In the first study of the program, aqueous solutions of 2,4-meta-xylidine hydrochloride were fed by stomach tube to Osborne-Mendel rats at 150/mg/kg/day (1/6 M.L.D.). The urine yielded a conjugated aromatic amino carboxylic acid as one of the major metabolites, a result similarly obtained upon feeding FD&C Red No. 1 dye. This change by the animal is an oxidation of a methyl group similar to that reported by Boyland and Sims (Biochem. J. 73: 377, 1959) for the conversion of 3,4-dimethylaniline (3,4-ortho-xylidine) to 4-amino, 2-methyl benzoic acid in rats.

Pharmacology

10

ACTIVE TRANSPORT OF URACIL ACROSS THE INTESTINAL EPITHELIUM. Dominick J. Tocco* and Lewis S. Schanker. Natl. Insts. of Health, Bethesda, Md.

An earlier study of the intestinal absorption of some pyrimidines showed that uracil and thymine cross the intestinal epithelium by a combination of two processes: active transport; and passive diffusion (J. Pharmacol. and Exper. Therap., in press). The present report describes some of the characteristics of the pyrimidine transport mechanism in the rat. Sacs of everted small intestine were filled with saline solutions containing 0.02 mmol/l of uracil-2-C^{14}. The sacs were suspended in the same solution and incubated aerobically at 37°C. After 1 hour, the mucosal and serosal solutions were collected and the concentration of uracil measured. The rat intestine transported the pyrimidine, from the mucosal to the serosal side, against a concentration gradient. Under anaerobic conditions, active transfer of uracil did not occur. Uracil transport was markedly depressed by 1 mmol/l of various metabolic inhibitors such as iodoacetate, 2,4-dinitrophenol, p-chloromercuribenzoate or phlorizin. The active transfer of uracil was also blocked by 0.5 mmol/l of thymine or hypoxanthine, and was slightly depressed by 5 mmol/l of 6-azathymine or 6-azauracil. Studies with the hamster and frog indicate that uracil is actively transported across the intestines of these species.

Pathology

11

ELECTRON MICROSCOPIC APPEARANCE OF LIPIDS IN JEJUNAL MUCOSA. Wilbur A. Thomas and Robert M. O'Neal. Dept. Pathology, Washington U., St. Louis, Mo.

Rats were fasted for 24 hours and subsequently given 10 ml. of melted fats by stomach tube. Animals were killed at intervals, usually one hour, after the fat feedings, and the upper jejunal mucosal cells examined by electron microscopy of osmium-fixed thin sections. Osmophilic intracellular masses, representing absorbed lipid, were found in all rats after 1 hr. The lipid masses were homogeneous and very electron dense in rats fed the highly unsaturated fats, corn oil and cottonseed oil. But in rats fed highly saturated or hydrogenated fats, butter and Crisco, the masses were not as osmophilic and appeared as irregular or laminated forms with clear areas between lipid layers. The degree of osmium fixation of the lipid masses, as shown by the proportion of electron-dense material in the fat, probably indicates degree of unsaturation of the lipid. Fasted control rats, as well as those fed water or cholesterol, had few or no lipid masses in the absorptive cells of jejunal mucosa. (Supported by a USPHS grant from the National Heart Institute). Dr. Thomas' present address: Albany Medical College, N.Y.

Pathology

12

ABSORPTION OF L-PHENYLALANINE BY EVERTED INTESTINAL SACS. R. P. Spencer and A. H. Samiy (Introduced by S. Warren). Cancer Research Institute of New England Deaconess Hospital, Peter Bent Brigham Hospital, and Department of Biological Chemistry, Harvard Medical School, Boston, Mass.

With the same initial concentration of L-phenylalanine on both sides, several parameters of absorption were studied using everted sacs of hamster small intestine. At equilibrium (one hour), in terms of μmoles transported/100 mg tissue (Δ), absorption was not uniform with length, but was least in end segments and greatest in midgut. As a function of final L-phenylalanine concentration on the mucosal side (2 to 20 μmoles/ml), Δ was described by a Langmuir adsorption isotherm ($\Delta = ac/K+c$); where c is concentration, a is capacity and K is an equilibrium constant. These constants could be evaluated for both end and midgut segments. The concentration ratio, aside from establishing the fact of absorption, was not found to be a useful measurement. Using a different parameter (the concentration gradient: $C_{serosal}-C_{mucosal}$) as a function of $C_{mucosal}$, data could be described by a Lineweaver-Burk plot which allowed evaluation of transport maxima and equilibrium constants for both end and midgut segments. (Supported in part by the Helen Hay Whitney Foundation and the Atomic Energy Commission).

XUM

13 *Nutrition*

SHORT TERM RADIOISOTOPE STUDIES OF INTESTINAL GLUCOSE ABSORPTION IN THE RAT. J. Martyn Bailey*, Joseph H. Roe, and Choong H. Woo*. Dept. of Biochemistry, George Washington Univ. School of Med., Washington, D. C.

The rate of glucose absorption and the free glucose and phosphate ester content of the intestinal mucosa were measured as a function of the glucose concentration in the lumen. A twenty five-fold increase in the glucose concentration in the lumen resulted in only a two-fold increase in the mucosal phosphate ester content. With 0.1% glucose in the lumen, the turnover time of the mucosal glucose pool during in vivo transport was approximately 30 seconds. The glucose and phosphate esters in the mucosa were analyzed following brief (10 seconds) exposure of the lumen to glucose-U-C^{14}. Three per cent of the total radioactivity was found in the phosphate esters and 85% in the mucosal glucose. About 10% was present in compounds which have not yet been identified. The specific activities were in the ratios: mucosal glucose (100), glucose-6-PO_4(17), glucose-1-PO_4(13), fructose-6-PO_4(11) and fructose-1,6-diPO_4(3). If the mucosal glucose is considered as glucose in process of transport, the results are inconsistent with the theory that phosphate esters are obligatory intermediates in this transport process in the rat.

14 *Nutrition*

FACTORS AFFECTING THE ABSORPTION OF METHIONINE FROM THE INTESTINE OF THE CHICKEN. M. Wight Taylor and Sam Yankelowitz*. Dept. of Ag. Biochemistry, Rutgers, The State University of N. J., New Brunswick, N. J.

Using the Thiry-Vella fistula in the chicken (Newman, H. J. and M. W. Taylor, Am. J. Vet. Res. 19, 473, 1958) various factors affecting the rate of absorption of methionine were studied. Confirming earlier work (Paine, C. M., H. J. Newman and M. W. Taylor, Am. J. Physiol., 107, 9, 1959) it was found that L-methionine is absorbed at nearly twice the rate of the D- isomer. Absorption of D-methionine occurred at nearly a constant rate over a period of an hour whereas the rate for the L-compound rose to a plateau from 15 to 50 min. and then dropped off. DL-methionine showed intermediate values. Following a 20 min. rest period the cycle could be repeated. With flow rates varying from 0.6 to 3.0 ml per min. the rate of absorption of both D- and L-methionine varied inversely, though not linearly, with flow rate. The effect of varying both concentration of methionine and flow rate will also be reported and the effect of the presence of other amino acids, including ethionine. It is suggested that the rate limiting factor in absorption of L-methionine is the passage of the amino acid from the site of adsorption in the intestinal epithelium through the intestinal wall to the blood stream.

15 *Immunology*

EFFECT OF SODIUM DEHYDROCHOLATE AND NIACIN ON CHOLESTEROL ABSORPTION. C.S.Narayana Setty (intr. by H.C. Batson), Univ. Illinois College of Med., Chicago, Ill.

It was found that sodium dehydrocholate would not substitute for the obligatory action of cholic acid in the absorption of cholesterol. It was thought that 300 mg daily of dehydrocholate in the diet might compete with or block the action of the 25 mg of taurocholate normally secreted by a 225 gm rat into the intestine. So, using cholesterol-4-C14 as a tracer, cholesterol absorption was studied in 200-225 gm rats of the Sprague-Dawley strain, with 300 mg of sodium dehydrocholate in the diet. The feces were fractionated, as per Siperstein et al into (1) unsaponifiable, (2) unsaponifiable nondigitonin precipitable, and (3) the bile acid fraction. No change occurred, except that the dehydrocholate decreased the bile acid fraction. Niacin has been reported to be hypocholesterolemic in man. Might niacin increase the bile acid or one of the other fractions? Adding niacin at a level of 1% in the diet was without effect.

126. GASTROINTESTINAL TRACT—Liver

1 *Physiology*

SOME EFFECTS OF COLCHICINE ON LIVER. Lola S. Kelly, Donner Laboratory, University of California, Berkeley.

In the course of studies on the effects of colchicine on desoxyribose nucleic acid (DNA) metabolism in mice, it was noted that there was a decrease of DNA synthesis in many tissues. In contrast, liver DNA synthesis rose markedly. One day after 0.03 mgm colchicine 2-hour liver DNA P^{32} specific activities were approximately ten times normal. H^3 thymidine autoradiographs demonstrated that increased DNA synthesis occurred in both parenchymal and littoral cells. The appearance of mitotic figures in the liver following colchicine treatment has been reported previously by several investigators. To determine whether this proliferation in the liver was a response to injury, two liver functions were tested. The sleep time, following a standard dose of Nembutal, which presumably tests parenchymal cell function, was somewhat prolonged. A very pronounced depression in the rate of removal of intravenously injected colloidal carbon demonstrated impaired reticuloendothelial system function.

2 *Physiology*

HEPATIC CONJUGATION AND EXCRETION OF PHENOLSULPHONPHTHALEINS. Agamemnon Despopoulos and Charles W. Gross*. Department of Pharmacology, University of Louisville, School of Medicine, Louisville, Ky.

Hepatic metabolism of phenolsulphonphthalein dyes was examined by clearance techniques in isolated perfused livers of Wistar rats. Each dye appeared in the bile within two minutes of its addition to the perfusate. The clearance of chlorphenol red (CPR) which is representative of these compounds, decreased with time. A metabolite of CPR, tentatively identified as an ethereal sulfate, was isolated from bile, its excretion increasing with time. The sum of the two components in the bile was constant. The data suggest that CPR and its metabolite compete for active excretion in the liver cells. (Supported by a grant from the Owensboro - Daviess County Heart Association, Ky.)

3 *Physiology*

HEPATIC CLEARANCE OF INDOCYANINE DYE. John Kampine*, W. J. Stekiel* and James J. Smith. Dept. of Physiol., Marquette Univ. Sch. of Med., Milwaukee, Wisconsin.

Wheeler et. al (Proc. Soc. Exp. Biol. 99:11, 1958) reported that nearly 100% of an injected dose of indocyanine dye was recovered from the bile. They also demonstrated that it does not appear in the urine and that intestinal absorption is minimal. Earlier studies in this laboratory indicated that its disappearance from the blood followed first order kinetics and that its initial distribution was limited to the plasma compartment. The dye satisfies most of the requirements for use in hepatic clearance studies. In the present study with dogs the plasma clearance rate for indocyanine was significantly reduced by hepatectomy. However, the plasma-dye concentration continued to fall, suggesting some extra-hepatic removal of indocyanine. Plasma clearance curves for the i.v. injection of 1 mg/kg in normal human subjects were compared with those from patients with abnormal hepatic function tests. Clearance rates in the patients were markedly decreased, suggesting that the plasma clearance of indocyanine dye may be useful as a hepatic function test.

4 *Pharmacology*

LIVER CELL PROLIFERATION IN X-IRRADIATED RATS AFTER SINGLE AND REPETITIVE PARTIAL HEPATECTOMY. George F. Leong*, Rita L. Pessotti*, and Ralph W. Brauer. U.S. Naval Radiological Defense Laboratory, San Francisco 24, Calif.

Following exposure to X Rays, abnormal mitoses occur in regenerating liver. Rat liver regeneration has been studied under repeated stimuli to proliferation to determine the regenerative behavior of a tissue containing cells with demonstrably damaged nuclear-mitotic apparatus. Irradiation involved two 400 r exposures (250 KVP X Ray whole body) three weeks apart. Successive partial hepatectomies were performed 8 weeks, 11 weeks and 14 weeks after the last exposure. Liver weights recovered to within 15 - 20 per cent of controls after the first, as well as after subsequent partial hepatectomies. Radiation exposure does not affect liver weight to body weight ratios under those conditions. By contrast, mitotic index in the irradiated rats is consistently 50 to 60 percent below values in non-irradiated controls. Less than 10 percent of the mitoses in regenerating liver of non-irradiated rats were visibly aberrant, whereas 75 to 85 percent of the mitoses in all post-radiation animals were abnormal. The discrepancy between mass regeneration and mitotic frequency, and the effect of a very high proportion of cells with damaged mitotic-nuclear apparatus on growth potential will be discussed.

5 *Pharmacology*

DIRECT MEASUREMENT OF THE BLOOD PRESSURE OF INTRALOBULAR BLOOD VESSELS IN THE RAT LIVER. Ralph W. Brauer, K. Nakata* and George F. Leong.* U.S. Naval Radiological Defense Laboratory, San Francisco 24, Calif.

Among key factors regulating blood flow distribution and fluid balance in the liver are the pressure relations in afferent and efferent vessels. While portal vein, hepatic artery, and hepatic veins are readily amenable to conventional techniques of pressure measurement, the actual pressure gradients in the sinusoidal circulation have not been reported to date. Improved transillumination techniques, together with micromanipulator guided microcanulae were used to determine blood pressures in minute vessels proximal and distal to the sinusoidal bed in the rat liver margin. Simultaneous determination of pressures in portal vein, and in the vena cava at the levels of entry of hepatic veins from the several liver lobes permit the construction of a pressure gradient diagram. In the pentobarbital anesthetized rat a surprisingly large pressure drop was found between portal vein and terminal distributing venules (from 12.5 cm to 6.5 cm H_2O rel. to hilum); a considerable pressure drop exists between portal venules and central veins (6.5 cm to 2.8 cm), and a negligible pressure drop between central vein and vena cava (2.8 cm to 2.3 cm in left lateral lobe).

6 *Pathology*

RELATION OF CHOLINE DEFICIENCY TO HEPATIC LIPOSIS AND MYOCARDIAL DAMAGE IN MICE. W. Lane Williams, University of Mississippi School of Medicine, Jackson, Miss.

Young adult albino mice were fed high fat-low protein diets for 1 to 10 months. After 4 months of restriction to a choline-deficient diet livers of all mice showed extreme liposis with formation of fatty cysts and of ceroid pigment. Supplementation with choline prevented the usual hepatic responses to choline deficiency except a restricted amount of cytoplasmic liposis which did not distort the parenchymal cells or involve all zones of lobules. In choline-deficient mice the incidence of myocardial injury (fibrosis of limited extent) was 23.6% during 9-40 weeks of feeding the diet. During the same period 66.6% of the choline-supplemented mice had lesions showing active myocardial necrosis or extensive fibrosis or both. In choline-deficient and in choline-supplemented mice other organs including kidneys and blood vessels were free of significant lesions. The data indicate that the choline deficiency is not a specific cause of myocardial damage and that supplementation with choline at a level sufficient to prevent extensive hepatic liposis increases injury to the heart.

7 *Pathology*

THE FATE OF LIVER CELLS. G. W. H. Schepers, M.D., D.Sc. Haskell Laboratory, E. I. du Pont de Nemours & Co., Wilmington, Delaware.

Past and current concepts concerning the structure and function of the liver presume a static histoarchitectonic organization, uniformly mediating polyphasic functions through each hepatic cell. These theories are challenged by cytological and histochemical evidence emanating from liver responses to specific classes of toxic chemicals. These compounds modify the rate and pattern of mitosis, selectively retard or enhance cell maturation and accelerate or delay their final dissolution. The hepatic cord is conceived of as an escalator system originating in the paraportal zone and ending at the centrilobular vein. New cells are generated paraportally. Here metabolism is in abeyance. As the cells shift medially, they commence to participate in glycogen, protein, fat and bile synthesis. Functions are selectively modified according to the position occupied by individual cells along this shifting base. Degenerative processes appear in the older centrilobular cells. As the cells approach the hepatic vein, they are finally eliminated through autolysis or bodily migration into the hepatic sinuses. Deciduous hepatocytes are demonstrable in the inferior vena cava and the lung.

8 *Pathology*

MECHANISM OF HEPATIC FIBROSIS. H. Popper, F. Schaffner and F. Paronetto*. Depts. of Path. & Med., Mt. Sinai Hosp., New York.

Liver biopsy and autopsy specimens from patients without and with liver disease and from experimental animals with rapid fibrogenesis were examined routinely, in ultrathin sections in visible light, and by electron microscopy. Fiber accumulation results from collapse, new formation or from both. It occurs in portal tracts, within parenchyma around damaged liver cells or around proliferating ductules, the latter particularly in diffuse fibrosis of subacute ethionine intoxication. In portal tracts fibrogenesis is usually associated with fibroblasts best seen in rapid local fibrogenesis after intrahepatic carrageenin. Around liver cells, single fibers but no basement membranes are noted. In damage fibers increase in tissue spaces without conspicuous fibroblasts but usually with proliferation of Kupffer cells. This differs from framework duplication after loss of cell plates (flat collapse). Fibrosis forms outside basement membranes of proliferating ductules. This is the main pathway to fibrosis in various human and experimental cirrhoses. Electron microscopically no difference exists between collagen and reticulum fibers. No fibrils could be demonstrated in cell cytoplasm. Altered hepatic cells and proliferated ductules seem to act as a stimulus around which fibers are laid down. In most types of cirrhosis, fibrosis is secondary to alteration of parenchyma. (Supported by Army Contract DA-49-007-MD-790).

9 *Pathology*

ELECTRON MICROSCOPY OF THE HEPATIC DUCTULAR CELL REACTION. F. Schaffner and H. Popper. Depts. of Medicine and Pathology, The Mount Sinai Hospital, New York, N.Y.

Characteristics of interstitial hepatic cells called oval or ductular cells found in experimental injury were compared by electron microscopy with human bile ductules. They had similar features like fine microvilli projecting into a lumen and a thin structureless and continuous basement membrane surrounding even irregularly proliferating ductules. They contained fewer and smaller mitochondria than liver cells and irregular endoplasmic reticulum. Transitions between liver and ductular cells were so far not seen. Differences in cytoplasmic characteristics between experimental conditions and human disease were noted. Periductular fibers varied from a few bundles of fibrils to a thick envelope of bundles always outside the basement membrane. Lymphatic and blood vessels were always closely approximated to ductules. Mononuclear cells were scattered between ductules and vessels. The most active ductular proliferation when ductules were not dilated was associated with the most periductular fibrosis, when mesenchymal cells were present. Ductular structure suggests that ductules play a role in a metabolic fashion as far as bile formation is concerned possibly related to the concentration of bile and that ductular cell proliferation is an effort to excrete toxic substances. (Supported by USPHS Grant A-3088).

XUM

10 *Pathology*

QUANTITATIVE FEATURES OF DUCTULAR CELL REACTION. A.Rubin, F. Hutterer & E.J.Singer (intr.by H.Popper). Mt.Sinai Hosp., N.Y.

Ethionine intoxication was produced in rats,with modifications by simultaneous methionine,recovery diet,and cortisone. For comparison bile duct ligation and high fat/low protein diet were used.Cell counts were performed,and DNA,RNA,protein,and hydroxyproline were determined.By the 7th week hepatic cells decreased from 58% to 11%,ductular cells increased from 6% to 72%,and mesenchymal cells dropped from 36% to 19%.At the same time the liver weight nearly doubled,but the DNA increased 5X. This suggests that the total number of ductular cells increased 75-100X while the total number of mesenchymal cells increased 3-5X,with only slight loss of hepatic cells.After 2 weeks recovery the ductular cells fell to 16%,illustrating the extreme lability of the ductular cell reaction.With simultaneous methionine the ductular cells rose only to 10% and with cortisone remained at 5%.By the 7th week the total protein of the liver increased by 60%,but the rate of increase of DNA was 460%,indicating a high nucleus:cytoplasm ratio for proliferating cells,which corresponds to ductular cells.The increase in hydroxyproline paralleled the rise in DNA.In all animals the hydroxyproline:DNA ratio remained remarkably constant.The ductular cell proliferation was always accompanied by fibrosis,but in view of the increased mesenchymal cells,it is not established which cells are responsible for fiber formation.

11 *Pathology*

Centrolobular stainable lipid in livers of mice with gold-thioglucose-induced obesity. Hs Imai, JS Meyer and HW Scott (intr. by WS Hartroft) Pathology, Washington University.

Obesity induced by hypothalamic damage in rats is associated with accumulation of large amounts of stainable periportal hepatic lipid(Meyer and Hartroft 1959). To explore further the relationship between obesity and hepatic fat, young adult mice of CFW were made obese by injection of gold-thioglucose. They were fed ad libitum and survivors killed after 2 months. The results indicated that this form of obesity is associated with centrolobular accumulation of a hepatic lipid. The fatty accumulation could not be explained on the basis of a toxic effect of gold-thioglucose. Mice became obese only after single doses,and non-obese mice receiving large doses in divided weekly amounts showed less total and centrolobular hepatic lipid than obese mice. Mice examined within 4 days after injection showed no significant centrolobular hepatic fat. We conclude that the centrolobular accumulation of hepatic lipid was directly associated with the obesity. Reasons for the difference in lobular distribution of hepatic lipid in mice with gold-thioglucose-induced obesity and in rats with hypothalamic obesity are unknown. The consistent centrolobular localization of fat again emphasizes the metabolic heterogenity of the hepatic lobule. (supported by a grant from Lipotrope Foundation)

12 *Pathology*

CIRRHOSIS OF POSTNECROTIC TYPE IN CHOLINE-DEFICIENT RATS. W. Stanley Hartroft and Joseph W. Grisham* Department of Pathology, Washington University School of Medicine, St.Louis 10,Mo.

Cirrhosis in choline-deficient rats has been considered 'Laennec'(Monolobular). But under some conditions, lesions may mimic grossly and microscopically 'postnecrotic'(multilobular) cirrhosis of man. The latter type may occur in animals whenever experimental conditions favor extensive parenchymal regeneration: - rats maintained on deficient diets (particularly high protein but relatively low methionine) for long periods, or rats restored to normal diets after development of 'Laennec' cirrhosis. In these livers, multiple portal and central areas are found in sections of grossly apparent macronodules. Between them, relatively wide trabeculae appear to have resulted from coalescence of several narrow ones from compression by macronodules. This observation supports the concept that nutritional abnormalities in man (e.g. alcoholism) may sometimes result in a 'postnecrotic' cirrhosis even in the absence of episodes of frank necrosis induced by ischemia, toxins or infections. (Supported by a grant-in-aid from the USPH, No. C-2548).

13 *Pathology*

HISTOPATHOLOGIC CHANGES FOLLOWING FERROCENE (DICYCLOPENTADIENYL-IRON) ADMINISTRATION. R. J. Stein, G. Kent* and J. D. Taylor. Div. of Exp. Therap., Abbott Labs., No. Chicago, Ill. and *West Suburban Hosp., Oak Park, Ill. (intr. by J. de la Huerga).

Ferrocene (F) has properties of an "aromatic" character. Rats fed F in daily doses of 250 mg/kg or over died within 3 days. Large deposits of hemosiderin were present in hepatic parenchymal cells and in RE cells of the spleen. Other rats were kept on daily doses ranging from 1 mg to 200 mg/kg and were sacrificed after 1, 2, 4, 8 and 12 weeks. With increasing amounts of F administered, correspondingly larger deposits of hemosiderin were present in the liver cells, spleen, adrenal cortex, lymph nodes, and kidneys in that order. The hemosiderin in the liver was located predominantly in the lobular periphery. In later stages these areas exhibited conspicuous hepato-cellular degeneration and regeneration, and phagocytosis of iron by macrophages. Ductular cells and new fiber formation were not observed. These features were even more pronounced in mice where a slight increase in reticulum fibers about the portal fields was noted after 4 weeks. The morphologic features in the later stages suggest a relationship of the hepato-cellular damage to iron overload. Except for lack of a ductular cell reaction and fibrosis the changes resemble those seen in early human hemochromatosis.

14 *Pathology*

INDUCTION OF LIVER-CELL TUMORS IN THE HAMSTER BY CARBON TETRACHLORIDE. B. Terracini and G. Della Porta (intr. by P. Shubik). Division of Oncology, The Chicago Medical School, Chicago, Ill.

Male and female Syrian golden hamsters received 6.25-12.5 microliters of carbon tetrachloride by stomach tube weekly for 30 weeks. All the animals surviving at least 3 months after termination of the treatment developed benign or malignant liver cell tumors, one with metastases. Portal fibrosis and regenerative nodules occurred commonly but no cholangiomatous tumors were observed. This contrasts sharply with our other finding that 2-acetylaminofluorene induced only cholangiomatous tumors in this species. The biological differences between various species in their response to liver carcinogens and the histogenesis of liver tumors will be discussed.

15 *Pathology*

EARLY CHANGES IN THE FINE STRUCTURE OF THE HEPATIC CELL IN ETHIONINE-FED RATS. J.W. Grisham (intro. by W.S. Hartroft) Dept. of Pathology, Washington University, St. Louis, Mo.

Within a week after male rats (100-150 gm.) are started on an adequate diet containing 0.25% DL-ethionine, certain hepatic cellular organelles exhibit changes in electronmicrographs not seen in controls pair-fed the same diet without ethionine. In experimentals, parallel profiles of ergastoplasm are abnormally sparse with only occasional strands often encircling mitochondria. Although ribonucleoprotein (RNP) particles are still applied to remaining parallel arrays many particles lie free in the cytoplasm. Particulate glycogen is greatly decreased. Smooth-walled vesicles not associated with RNP particles are prominent. Pathologic lipid droplets unrelated to any cytoplasmic organelle are numerous along the sinusoidal border. Nuclei and nucleoli greatly enlarge and the nuclear membrane is often infolded with invaginations frequently containing lipid. Mitochondria are not distinctly altered but cytosomes or "microbodies" are numerous. Interrelationships of hepatic cells are not changed and other cellular elements are normal, although bile ductular cells proliferate. The similarity of these changes to those reported by others (Porter and Bruni, 1959) in rats fed an azo dye is striking and suggests a common pathway. (Supported by a grant from the Lipotropic Foundation).

16 *Pathology*

A QUANTITATIVE ELECTRON MICROSCOPIC APPROACH TO CENTROLOBULAR HEPATIC ALTERATIONS IN INITIAL STAGES OF CHOLINE DEFICIENCY IN RATS. E.A.Porta and J.S.Meyer (intr. by W.S.Hartroft). Department of Pathology,Washington University Sch.Med., St. Louis, Mo.

Changes produced in livers of rats by 4 days of choline deficiency are adaptable to electron microscopic study. Male Wistar rats (150-200 gms) were fed a choline-deficient diet and controls the same diet supplemented with 0.5% choline Cl or a standard commercial ration. All rats were killed after 4 days and centrolobular hepatic cells studied by light and electron-microscopy. Surface areas of 5 cells in each animal were determined and numbers of their mitochondria in electron-micrographs counted. Ratios of oblong to round mitochondria, electron-dense areas and other alterations were established. Statistical analyses of numerical results indicate that at 4 days the average number of mitochondria is the same for the 3 groups but swelling of mitochondria became significant in the choline-deficient rats. These studies suggest that dietary choline may act to preserve normal shape, size (and possibly function) of hepatic mitochondria. (Supported by Grant USPH No. C-2548).

17 *Pathology*

ACID PHOSPHATASE AND RETICULOENDOTHELIAL SYSTEM. T. Barka*, P. Schaffner & H. Popper. Dept. Path., Mt. Sinai Hosp., N.Y.

Reticuloendothelial (RE) cells (Kupffer cells, macrophages and reticular cells of spleen, thymus and lymph node) are characterized by a high acid phosphatase activity, demonstrated by a modified azo-dye method. Enzyme activity is localized in organelles (phagosomes) which store vital dyes, supporting the relation of phagosomes to lysosomes. Changes of acid phosphatase activity of liver, spleen, thymus and lymph node of rat were followed chemically and histochemically in 3 conditions known to alter phagocytic activity of RE cells, namely, repeated injection of trypan blue and single injections of foreign protein or typhoid vaccine. These caused variable increases of acid phosphatase activity expressed both on wet weight and DNA basis. Greatest changes were found in thymus and lymph node, where acid phosphatase activity rose to 2-3X. Histochemically, increased acid phosphatase activity was generally associated with activation and proliferation of macrophages or reticular cells. Histochemical demonstration and determination of acid phosphatase offers another parameter of measuring activity of RE cells. (Supported by USPHS Grant A-1783).

18 *Pathology*

MITOCHONDRIAL CHANGES IN ETHIONINE INTOXICATION. F. Hutterer & V. Fitschen (intr. by H. Popper). Dept. of Pathology, Mt. Sinai Hosp., New York, N.Y.

Ethionine,15mg/100g body weight,produced fatty liver in female rats. Mitochondrial oxidation of stearate(SO),glutamate (GO),proline(PrO),and oxidative phosphorylation(PO) decreased only at a dosage of 75mg/100g. A transient rise of total serum fatty acids was followed by a rise in liver fat,starting 6 hours after injection,and by a decrease of PrO and PO starting at 12 hours. Irrespective of fat accumulation PrO and PO decreased similarly in both sexes,and simultaneous methionine injection prevented mitochondrial changes and fatty infiltration. Low ethionine doses with dinitrophenol or Janus Green produced mitochondrial damage without increasing fat; simultaneous cortisone increased liver fat without decreasing PrO,SO, GO. An 0.5% ethionine diet increased liver fat during the first 4 days; it gradually returned to normal by the 3rd week. Addition of ATP and DPN in vitro corrected the decrease of SO & GO in the 1st week,but was ineffective by the 3rd week. Increased liver fat does not necessarily produce detectable mitochondrial damage,and decreased mitochondrial function need not result in fatty liver. Mitochondrial damage and fatty infiltration seem to be independent effects of ethionine intoxication. (Supported by Army Contract DA-49-007-MD-790).

19 *Pathology*

DIETARY METHOD FOR INDUCTION OF CIRRHOSIS AND OBESITY IN RATS. George F. Wilgram and Dwight J. Ingle. University of Chicago and Tufts University Medical School Boston.

In order to gain some insight into the problem of hepatic lipid accumulation and the development of cirrhosis, 68 adult (av. 280 g.) male Sprague Dawley rats were put on the following dietary regimens: 22 animals were force-fed a diet which contained 65% mixed fats, 12% egg albumin and 7.5% yeast. Minerals and vitamins including choline were adequately supplied. Twenty-two animals were force-fed the same diet supplemented with 2% cholesterol. Twelve control rats ate chow ad lib. and twelve controls were given the experimental diet ad lib. The controls on chow were perfectly healthy, while the ad-lib.-fed rats on the experimental diet had a fatty liver of the periportal type but no cirrhosis. The force-fed rats, killed at intervals of three weeks, had doubled their weight after three months. All cholesterol-fed rats had severe cirrhosis. The important finding was the development of cirrhosis by force-feeding a diet free of cholesterol, high in fat, but "theoretically" adequate in respect to protein, vitamins and minerals.

127. GASTROINTESTINAL TRACT—Liver—Bile

1 *Physiology*

EXCRETION OF C^{14}-HYDROCHLOROTHIAZIDE IN THE BILE AND PANCREATIC JUICE OF DOGS. Elmer B. Pratt and Jerry K. Aikawa.* Dept. of Med., Univ. of Colorado Sch. of Med. Denver, Colo.

The excretion of C^{14}-hydrochlorothiazide (A) which is not metabolized in experimental animals was studied in 4 nembutalized dogs with acute biliary and pancreatic duct fistulas. Samples were collected for 1 hour periods after intravenous injection of secretin (20 units) and then secretin and A (1mg/kg). Bile, pancreatic juice and serum specimens were analyzed for Na, K, Cl, CO_2, pH, and radioactivity. Typical secretin effects on pancreatic juice (increase in volume and in CO_2) were not altered by A. Chemical measurements of bile were not altered by secretin alone or with A. The mean concentrations of A in bile at 20, 40 and 60 min. were 163, 208, and 65 mcgm/ml, respectively. The highest concentration of A in urine occurred in the first 40 min. (mean 197 mcgm/ml). The highest mean serum concentration of A was 1.53 mcgm/ml at 20 min. 4.3% of the injected dose was recovered in bile and 39.3% in urine. Pancreatic secretion of A was negligible. When A alone was given to an unanesthetized intermittent-type bile fistula dog, similar results were obtained. After injection, A produced no change in bile volume or composition although its concentration in bile was as high as in the urine. (Supported by Colo. Heart Assoc., U.S. Army DA-49-007-MD-479, U.S.P.H.S. H-847 grants.)

2 *Nutrition*

THE EFFECT OF NEOMYCIN (NEO) ON THE EXCRETION OF BILE ACIDS IN HUMANS. James G. Hamilton*, O. Neal Miller and Grace A. Goldsmith, Tulane Univ. Sch. of Med. New Orleans, La.

NEO lowers serum cholesterol in humans but elevates it in rats. Antibiotics administered to rats causes a decreased excretion of bile acids. In humans, NEO increased excretion of non-conjugated bile acids (silicic acid column purification and titration method). An ethanol extract was made of an acidified, lyophylized, 4 day feces sample from each a control period and one in which NEO was administered. Bile acids were extracted from the ethanol extract with ether and further purified by precipitating pigments with barium acetate-barium hydroxide and separating neutral compounds from acids on an alumina column. The purified extracts were chromatographed in benzene-ethanol mixtures on glass fiber paper impregnated with sodium silicate and in benzene-ethanol and isooctane-acetic acid mixtures on silicic acid papers. Results indicated that administration of NEO caused changes in pattern of bile acid excretion. The major changes were a decreased excretion of deoxycholic and lithocholic acid and a large increase in cholic and an unidentified non-polar acid which is chromatographically similar to 3-keto-cholanic acid. Total bile acid excretion was increased several times over that of control.

128. GASTROINTESTINAL TRACT–Motility

1 *Physiology*

THE EFFECT OF CYANIDE ON THE ETHER-SOLUBLE Na AND K CONTENT OF RAT KIDNEY SLICES. John C. Vanatta. Physiology Dept. Univ. of Texas Southwestern Med. School, Dallas, Tex.

Fresh rat kidney slices 0.5 mm. thick were incubated for 1 hr. in bicarbonate buffer under 5% CO_2, 95% O_2 atmosphere. The tissues were extracted with alcohol, 1:1 ether-alcohol, and ether. The extracts were filtered, dried, and the residue reextracted with ether. This residue was purified, and the Na and K content then determined. The ether extracts of slices incubated in bicarbonate buffer containing no glucose, and 2% cyanide contained 1.16 ± 0.24 mEq. Na/kg. wet weight and 0.45 ± 0.12 mEq. K/kg. Slices incubated in buffer with 0.2% glucose contained 18.71 ± 1.25 mEq. Na/kg. and 6.01 ± 0.79 mEq. K/kg. All values are averages of at least 6 determinations on slices from the same animals incubated simultaneously. Concentrations of ether-soluble Na and K in fresh rat kidneys are 26.76 ± 1.03 mEq. Na/kg. and 12.72 ± 1.41 mEq. K/kg. The results are interpreted as indicating that cyanide either promotes the breakdown of the ether-soluble materials, or that it inhibits resynthesis in a system where there is normally a rapid turnover of the substances. The classical concept of cyanide as an inhibitor makes the latter more tenable. (Supported by a research grant H-1574 C-5 from National Heart Institute, USPHS)

2 *Physiology*

THE EFFECT OF INTRA-DUODENAL INSTILLATION OF OLIVE OIL AND HYDROCHLORIC ACID ON THE MOTOR ACTIVITY OF THE FULLY INNERVATED PART OF THE STOMACH AND HEIDENHAIN POUCH OF DOGS. Stanley H. Lorber and Harry Shay. Fels Res. Inst., Phila., Pa.

The purpose of this study was to investigate the influences of neural and hormonal factors on motor activity. Dogs equipped with an esophagotomy, gastric fistula and Heidenhain pouch were used. A small tube was passed into the duodenum through which test substances were introduced at the rate of 2 ml. per minute. Balloons were used to record motor activity. The instillation of 2 to 10 ml. of olive oil produced inhibition of gastric motor activity. Olive oil, in doses of 4 to 10 ml., also produced inhibition of motor activity in the Heidenhain pouch but the latent period in the pouch was longer. Two ml. of olive oil depressed but did not completely inhibit pouch motor activity. Acid was a weak inhibitor of motor activity. Twenty ml. of acid inhibited gastric motor activity but did not effect or only depressed the motor activity in the Heidenhain pouch.

3 *Physiology*

THE CORRELATION OF THE MOTILITY OF THE DISTAL PYLORIC ANTRUM, THE PYLORIC SPHINCTER AND THE PROXIMAL DUODENUM. Joe Moon*, H. Louckes and J.P. Quigley. Univ. of Tenn. Med. Units, Memphis.

Motility of the distal 2cm. of the pyloric antrum or the pyloric sphincter and the proximal 2cm. of the duodenum of trained, unanesthetized dogs was recorded by the modified inductograph technic (Fed.Proc.16:1957,82). The contraction of the pyloric sphincter is a component of the antral peristaltic mechanism (Fed.Proc.18:1959,95). The proximal duodenum exhibited two types of motility; 1) 2-4 sec. waves at a frequency of 16-18/min. During fasting (16-20 hrs.) the 2-4 sec. waves usually occurred only when the antrum and sphincter were rhythmically contracting and relaxing. Following a meal, the 2-4 sec. waves occurred at a constant frequency of 16-18/min. for 30-60 mins. of observation and the amplitude was increased approximately 20%. 2) 10-14 sec. waves upon which was superimposed 3-4 of the 2-4 sec. waves. The 10-14 sec. waves were a continuation of the antral-sphincter peristaltic wave. During fasting sometimes a 10-14 sec. wave followed every antral-sphincter wave and sometimes the antral-sphincter wave did not appear to go beyond the sphincter. Following a meal, the 10-14 sec. waves usually followed every antral-sphincter contraction with a frequency of 4-6/min. for 30-60 mins. of observation. Both types of duodenal wave were occasionally superimposed on slow tonus waves of 1-2 mins. duration. Sup. by U.S.P.H. Grant A1764C8.

4 *Physiology*

EFFECT OF INSULIN HYPOGLYCEMIA ON GASTRIC MOTILITY OF SPIDER MONKEYS. F. P. Brooks and S. S. Rothman*. Dept. of Physiology, Univ. of Pennsylvania, Philadelphia, Pa.

Gastric motility was recorded in 3 fasting unanesthetized spider monkeys with a small balloon in the antrum introduced through a gastric fistula. The balloon was filled with air and connected to a water-filled U tul` Recordings were made on a smoked drum. After an hour control period, 2 units/Kg of insulin was injected intravenously and recording continued for 3-4 hours. Blood sugars were 13-36 mg%. Single contractions of small amplitude and 5-10 secs. duration occurred at rates of 0-4 during the first hour. Contractions appeared 5-33 minutes after the insulin was given and continued for 2-3 hours. The fastest rate occurred during the first hour; 28-142. Most contractions were larger in amplitude than those during the control period. Their duration varied from 5-15 secs. (Supported by Grant No. RG-5007 C2, National Institutes of Health.)

5 *Physiology*

THE PRESSURE PROFILE OF THE GASTRODUODENAL JUNCTIONAL ZONE IN DOGS. Benno M. Brink* and Charles F. Code. Mayo Clinic and Mayo Foundation, Rochester, Minnesota.

This study was undertaken to determine whether a zone of elevated pressure between the stomach and duodenum might be detected in dogs by technics used for determination of pressure in other junctional zones. Cannulas were placed in the midregions of the stomach and duodenum of dogs trained to lie quietly in the supine position. Small pressure-detecting units were drawn from one cannula to the other through the junctional zone. When one of these was a balloon, 7 mm. in diameter, a narrow band of elevated pressure was encountered in 86 per cent of 44 tests done on four fasting dogs. Mean width and pressure of the band were 1.3 cm. and 12 cm. of water. While N/10 HCl was introduced into the duodenum the mean pressure in the junctional zone increased by 10 cm. of water above control levels. A similar rise of pressure occurred in the region 15 to 20 minutes after olive oil had been placed in the duodenum. The conclusion is drawn that when pressure was encountered during withdrawal of the balloon, the diameter of the canal through which it was being moved was less than the 7 mm. diameter of the balloon itself. The data allow no conclusion, however, as to whether the canal was actually closed.

6 *Physiology*

SEROTONIN CONTENT OF THE INTESTINAL WALL AND LUMEN OF THE RABBIT SMALL INTESTINE. T. Hayama (introduced by C. A. Finch) Dept. of Pharmacology, University of Washington School of Medicine, Seattle, Washington.

Intestinal movement clearly passes food towards the anus. A motility gradient is known to exist. Since serotonin (5HT) in the intestinal mucosa facilitates the peristaltic reflex, the possible correlation in the rabbit between 5HT released at different levels and the motility gradient was studied. 1. The 5HT content of the small intestinal wall was measured at six levels. In 3 of 8 experiments there was a slight decrease in 5HT content toward the colon, while in the others there was no gradient. 2. In order to measure the 5HT in the lumen content the ends of three 10 cm segments of small intestine from the duodenum, jejunum and ileum were connected to T tubes in rabbits. The T tube was connected to a Marriotte bottle and filled with Tyrode's solution at constant pressure. After 20 minutes the segments were surgically removed and the contents washed into a test tube. The 5HT contents of these samples were measured by Vene's method. There was a decrease in luminal content 5HT in the intestine as one progressed in a distal direction.

1 *Pharmacology*

IN VITRO ACTIONS OF STEROIDS ON SMOOTH MUSCLE. James A. Setliff* and Allan D. Bass. Dept. of Pharmacology, Vanderbilt Univ. School of Medicine, Nashville, Tenn.

We have previously shown that adrenocortical steroids decrease the normal muscle tone of isolated aged guinea pig ileum in doses of 0.005 μmoles/ml. and block or reverse the action of pilocarpine and other cholinergic drugs in isolated bronchial, uterine, and intestinal smooth muscle. Cortisone acetate completely blocks pilocarpine induced contractions of the isolated guinea pig ileum at a dose level (0.0176 μmoles/ml.) which has little effect on histamine induced contractions of the same amplitude (Surgical Forum IX: 624, 1959). However, this smooth muscle relaxing effect seems to be relatively nonspecific since 50% maximal contractions of the isolated guinea pig ileum induced by a variety of stimulating agents were blocked to the same extent by the same doses of steroid. In addition, the fact that a variety of steroids are effective in this system--testosterone, progesterone, estradiol, cortisone acetate, and desoxycorticosterone--indicates a relative nonspecific effect on smooth muscle. The blocking action of the steroids is readily reversible since washing restores the normal sensitivity of the gut to contractions induced by other drugs.

129. GASTROINTESTINAL TRACT—Pancreas

1 *Physiology*

A COMPARATIVE STUDY OF "JORPES-MUTT" SECRETIN AND "BOOTS" SECRETIN IN MAN. David C. H. Sun and Harry Shay, Fels Res. Inst., Temple Univ. Med. Center, Philadelphia, Pa.

A comparative study was done with "Jorpes-Mutt" secretin and "Boots" secretin on the serum enzyme response and external secretin of the pancreas in man. This was done to determine the relative activities of these two secretins and the best dose of "Jorpes-Mutt" secretin for a diagnostic test. Fasting patient, intubated with two Levine tubes, one in the stomach and the other in the duodenum, and continuous suction was used. After the collection of a 20-min. basal specimen, secretin was injected intravenously. Duodenal contents were collected in 10, 10, 20 and 20-min. specimens. Volume, pH, bicarbonate and amylase concentrations determined. Blood samples, taken at fasting, 1 and 2 hours after secretin were analyzed for serum amylase and lipase. Three to four dose levels of each secretin were studied in the same patient on different days. A dose response curve was obtained on volume and bicarbonate output of the duodenal contents. 0.05 mg. of "Jorpes-Mutt" secretin is approximately equal in activity to 80 clinical units of "Boots" secretin. Analysis of our data indicates that an excessive dose of secretin could induce abnormal serum enzyme elevation in normals. The probable mechanism will be discussed.

2 *Physiology*

Effects of Some Anticholinergic Drugs on Basal and Secretin-Stimulated Pancreatic Secretion in Man. David A. Dreiling* and Henry D. Janowitz. The Mount Sinai Hospital, New York, New York.

The interrelation of neural and hormonal factors in the regulation of pancreatic secretion in man remains unresolved. In the present study a series of newer anticholinergic drugs (JB 323, 340, 305 and 437 Lakeside) were given parenterally to seventy normal subjects and to thirty patients with inflammatory disease of the pancreas between tandem injections of secretin (1.0 units/kg). This resulted in a profound depression (up to 90%) of both basal and secretin stimulated secretion for these parameters: volume rate of flow, bicarbonate output, and amylase output. There were no significant changes in maximum bicarbonate concentration. The results are interpreted as indicating that the responsiveness of the human pancreas to secretin is dependent on functional innervation. These experiments furnish some direct evidence of the existence of a "primary" secretion by the pancreas of an isotonic solution of bicarbonate independent of rate of secretion.

3 *Biochemistry*

A LECITHINASE A IN DUODENAL CONTENTS OF MAN. William C. Vogel and Leslie Zieve (introduced by H. R. Gutmann) VA Hospital and Univ. of Minn., Mpls., Minn.

A lecithinase A was demonstrated in the duodenal contents of man. The release of fatty acids from lecithin was measured titrimetrically. The decrease in lecithin and equivalent increase in lysolecithin was determined independently by quantitative paper chromatography. The action of the enzyme was markedly increased in the presence of sodium deoxycholate and moderately increased by glycine, glutamic acid, arginine, or lysine. The optimum medium for the hydrolysis was a glycine-borate buffer at pH 8.5, and the optimum temperature was 55° C. Heating in this medium at 80° C. for 30 minutes did not inactivate the enzyme. The enzyme was partially inhibited by calcium, magnesium, oxalate, oleic acid, and diethyl ether, and nearly completely inhibited by EDTA.

4 *Pathology*

TOXIC NEPHROSIS IN RABBITS AFTER THE ORAL HYPOGLYCEMIC DRUG PHENFORMIN (DBI). S.S. Lazarus and B.W. Volk. Isaac Albert Res. Inst. of the Jewish Chronic Disease Hosp., Bklyn. and Dept. Path., Albert Einstein College of Med., Bronx, N.Y.

A study was carried out on the effect of the oral hypoglycemic drug Phenformin (DBI) on the morphology of liver, pancreas, muscle and kidney of the rabbit. For this purpose 25 mg./kg. of the drug was injected subcutaneously for up to 6 weeks. The daily morning blood sugar values were usually within normal range. No significant changes were observed in pancreas, liver or muscle. However, there were marked alterations in the kidney. These consisted of diffuse extensive vacuolization of tubular epithelium, tubular dilatation and cast formation leading to fibrosis of the renal parenchyma. The earliest changes were observed between the 5th and 8th day and became progressively accentuated after 2 weeks. At approximately 3 weeks of treatment there was fibrosis and eventual widespread replacement of renal parenchyma by connective tissue. Frequently inspissated PAS positive casts were noticeable within dilated tubuli. Histochemical studies revealed a decrease of glucose-6-phosphate dehydrogenase, lactic dehydrogenase and succinic dehydrogenase activity which, however, seems to follow the tubular changes and therefore appeared to reflect the cellular damage rather than a primary site of action of the drug. No significant glomerular changes were observed. It is believed that the severe damage to the tubular epithelium reflects increased concentration of Phenformin in the tubular lumina or the reabsorption of the drug by renal epithelium. The possible applicability of these changes to the human will be discussed.

5 *Pathology*

PANCREATIC HYPERPLASIA IN RATS INDUCED BY SOYBEAN MEAL DIETS. Wm. E. Ribelin, A. N. Booth, and D. J. Robbins*. Western Regional Research Laboratory[1], Albany, Calif.

Soybean meal is an inexpensive and generally satisfactory source of protein. When raw soybean meal was fed to rats for 22 days or longer as the sole source of protein, grossly visible enlargement of the pancreas occurred and growth was inhibited. Microscopically the pancreatic acini were seen to have lost their regular circular outlines but were jumbled and without distinct lumens. The basilar portion of the hyperplastic acinar epithelium was intensely basophilic and increased at the expense of the zymogenic portion. Mitoses were occasionally encountered. Acini still spherical enough to be measured were significantly smaller ($p=<0.01$) than normal controls, apparently due to squeezing by adjacent hyperplastic cells. Nucleoli were significantly larger ($p=<0.05$), nuclei appeared larger but were not significantly so. Pancreatic islets appeared unaffected. Feeding amino acid supplement restored body weight to normal but did not correct the pancreatic structure. The pancreatic factor is highly labile, being destroyed by heating during processing of the meal, and is unlikely to be of general nutritional significance.

[1]/Western Utilization Research and Development Division, Agricultural Research Service, U.S. Dept. of Agriculture.

XUM

6 *Pathology*

THE EFFECT OF TOTAL PANCREATECTOMY ON EXPERIMENTAL HEMORRHAGIC PANCREATITIS IN THE DOG. M.S.Rittenbury* and R.H.Egdahl. Surg. Res. Lab., Med. Coll. Va., Richmond, Va.

Production of acute hemorrhagic pancreatitis by a standard bile-salt injection method is fatal to 100% of dogs within 24 hours. The present studies were undertaken to determine the effect of total pancreatectomy performed at various time intervals following the induction of pancreatitis. A series of 8 control dogs with total pancreatectomy survived from 2-6 days with no supportive therapy. Eleven animals in which the pancreas was removed more than 28 minutes after the production of pancreatitis failed to survive longer than 24 hours. Those animals in which the pancreas was removed less than 28 minutes following induction of pancreatitis survived for 48 or more hours. These results indicate that a lethal factor or its precursors has been formed as a result of bile pancreatitis and that after 28 minutes the factor or precursor has reached a high enough concentration in the body to prove lethal even in the absence of the gland. Studies are underway to further define this "lethal" factor.

130. GASTROINTESTINAL TRACT– Stomach

1 *Physiology*

EFFECTS OF ALVEOLAR O_2 AND CO_2 ON GASTRIC SECRETION IN MAN. A. Naitove* and S.M. Tenney (intr. by J.S. Robb) Dept. of Physiology, Dartmouth Medical School, Hanover, N.H.

Gastric acid secretion was studied in two normal subjects at sea level and after one and six days of continuous residence at high altitude (14,246 ft.). An evaluation of the separate and interacting effects of alveolar CO_2 and O_2 tensions on gastric acid secretion was made possible by appropriate variations in the inspired mixtures. Exposure to the hypoxia and hypocapnia of high altitude resulted in a mean increase in free HCl acid secretion (mEq/min) to levels four times sea level controls. The same degree of hypocapnia at the higher O_2 tensions at sea level resulted in a net decrease. After six days at altitude there was a fall in secretory rate towards normal. Hypercapnia uniformly was associated with marked increases in secretory levels, being highest at altitude. The findings suggest that the level of gastric acid secretion was directly related to alveolar CO_2 tension and that for any given CO_2 tension, secretion was inversely related to alveolar O_2 tension. Data on blood CO_2 content offered further information on the role of CO_2, indicating that blood pH may play a minimal role in modifying gastric acid secretion. (Supported by U.S.P.H. and N.S.F.)

2 *Pharmacology*

EFFECT OF CORTICOSTEROIDS ON NON-DIALYZABLE SUBSTANCES OF THE GASTRIC JUICE. Anton L. Hitzelberger and George B. Jerzy Glass (intr. by Linn J. Boyd.) Dept. of Medicine, New York Med. Coll., New York.

Fifteen patients with arthritis received 150 mg dexamethasone or 1 g prednisolone during a 6-week period. In 10, the same treatment was repeated after 1-4 months to check reproducibility of data. Gastric basal secretion was collected before, and on the 10th, 21st and 42nd days of treatment. In each collection, free HCl, pH, pepsin, 'cathepsin' and paper electrophoretic pattern of gastric juice were determined. During steroid treatment of 15 cases, acidity rose markedly only in 2, 'cathepsin' in 3, and pepsin in 5. In 9 cases, electrophoregram of gastric juice showed a uniform increase in concentration of cathodic component X_2, and, in 6 of these, a marked rise also in that of component Y_2. In 4 of the latter subjects recurrent epigastric pains occurred during steroid therapy, but with no occult blood in stool and with normal G.I. series. The components X_2 and Y_2 are non-identified, high molecular weight peptides of high positive charges, usually present at high concentration in histamine-stimulated gastric juices of patients with hypersecretion or peptic ulcer. The occurrence of these substances under steroid effect in often anacid basal gastric secretion may point to a common denominator of gastric abnormalities in peptic ulcer and in steroid-treated patients. (Supported by a grant from Merck Sharp & Dohme Research Laboratories.)

3 *Pathology*

STUDIES ON THE ATROPHIC GASTRITIS IN DOGS INDUCED BY A PREPARATION OF HUMAN GASTRIC JUICE. W.O. Smith*, Merlin K. DuVal*, Walter Joel* and Stewart Wolf. Depts. of Med., Surg. and Path., Univ. of Okla. and Vet. Admin. Hosp., Okla. City.

Recent studies from this laboratory have shown that repeated intravenous injections into dogs of preparations of human gastric juice containing Brunschwig's HCl inhibiting factor induce a gastric lesion with the characteristics of atrophic gastritis. After as few as ten injections at biweekly intervals the orderly arrangement of the mucosal glands was disrupted with loss of parietal cells, thinning of the membrane and extensive fibrosis. The lesion has the appearance of the atrophic gastritis in humans often associated with gastric carcinoma. No lesions occurred in other parts of the gut or elsewhere in the body, and there was no anemia after nine months of atrophic gastritis. In dogs having Heidenhain pouches the atrophic gastritis lesion was always present in the innervated main portion of the stomach but failed to appear or was minimal in the denervated pouch. Parietal cells in the pouch were well preserved. Nevertheless, both pouch and main stomach failed to secrete HCl even after histamine stimulation. Current studies with vagotomized dogs and various other operative procedures are directed at further clarifying the relationship of innervation to the mechanism of the atrophic gastritis.

4 *Pathology*

Alterations in the Functions of the Stomach Produced by Local Hypothermia. W.O. Griffen, Jr.*, A. Castaneda*, D.M. Nicoloff* & O.H. Wangensteen. Univ. of Minn. Med. School, Minneapolis

Studies of the influence of hypothermia upon gastric function have shown the following: At temperatures below 10°C. gastric secretion is virtually inhibited. Peptic activity is abolished. Gastric secretion of vagal origin is markedly decreased by local hypothermia. Cold reduces the antral secretagogue response to foods to a minimum. Cold has little or no influence on acid inhibition. Local gastric hypothermia has been useful in assessing the intestinal phase of gastric secretion. Local gastric hypothermia has proved to be a useful agency in the management of massive gastric hemorrhage from a variety of conditions including: gastric and duodenal ulcer; esophageal varices of portal hypertension, whether owing to an intra- or extrahepatic block; and stress ulcer. At 10°C. or below, there is a 66% reduction in venous outflow, probably reflecting a similar reduction of arterial inflow. Local gastric cooling delimits sharply pyloric outflow owing to reduction in motility, thus permitting achievement of local gastric hypothermia by direct perfusion of the stomach in some types of massive gastric hemorrhage. (Supported by a U.S.P.H.S. Grant & The Donald J. Cowling Fund for Surgical Research).

5 *Pathology*

A TOPOGRAPHIC STUDY OF GASTRIC EPITHELIAL HYPERPLASIA ACCOMPANYING GASTRIC CANCER. Sheldon C. Sommers, Mass. Mem. Hosps. and Boston Univ. School of Med., Boston, Mass.

A series of 100 surgical specimens of stomachs removed for cancer has been studied topographically with multiple sections to determine the extent and types of epithelial hyperplasia in the uninvolved mucosa. Previously, a similar analysis of random single stomach sections from another series of autopsied gastric cancer cases had shown an epithelial hyperplasia of either superficial, deep or combined subtypes in a total of about 80% of the specimens. The present systemic mapping method confirmed the high overall incidence of associated epithelial hyperplasia and indicated that random blindly obtained biopsy specimens would have demonstrated the hyperplasia in over three-quarters of persons with gastric cancer. Gastric epithelial hyperplasia is concluded to occur frequently and extensively with gastric cancer and to have a close pathogenetic relationship to its development. (Supported by a grant from Mass. Div., American Cancer Soc.)

6 *Nutrition*

Cr^{51} ASSAY OF GASTROINTESTINAL BLOOD LOSS IN SUBJECTS TAKING SALICYLATES. Richard M. Watson and Richard N. Pierson, Jr., (intr. by Edward H. Reisner) Dept. of Medicine, St. Luke's Hospital, New York, N. Y.

Aspirin has been reported to cause significant gastrointestinal bleeding. To measure this effect, 90 normal volunteers (60 females) were injected with Cr^{51} labeled RBC and fed 600 mg. aspirin 4 times daily for 7 days. Fecal blood content for 4-day collection periods was determined from the fecal: blood radioactivity ratio. Average daily blood loss without aspirin was less than 1 ml. 70% of subjects showed a significant increase over this while taking aspirin. Average daily blood loss for the whole group taking aspirin was 4.8 ml. (6.3 for females, 3.4 for males). 10% had loss of over 10 ml./day. In subsequent study periods use of enteric coated aspirin and the more soluble compounds, calcium aspirin complex and choline salicylate, resulted in average daily losses of 2.0, 2.0 and 0.5 ml., respectively. Simultaneous ingestion of aspirin and milk or alkali was not associated with decreased rate of bleeding in subjects who had previously bled on aspirin alone. Day-to-day blood loss was determined in 12 subjects and varied widely. Bleeding was not accompanied by significant change in plasma prothrombin time, and was believed due to local irritant action of salicylates on the gastrointestinal mucosa.

131. GASTROINTESTINAL TRACT—Stomach—Secretion

1 *Physiology*

THE SIGNIFICANCE OF POTASSIUM IN GASTRIC SECRETION. F. Hollander. GE Res. Lab., Mt. Sinai Hosp., N.Y.

Previous work in this lab. showed that K-concentration, [K], in gastric secretion induced by single or tandem histamine injections varies considerably in both dog and man. In general, the [K]-time curve rises promptly above its pre-injection level to a peak, and then falls off more slowly - independently of acidity, vol.-rate of secretion, and [Na]. Peak values may be 3 or more times blood serum [K]. Some of the experiments of long duration in dogs gave minimum levels of 1 meq/L or less. These observations suggest a number of possible sites of origin of this gastric K. Of all these hypotheses, the best was found to be one based on a general histamine-induced release of K into interstitial fluid by various tissues - a process which may be reversed within one hour, and sometimes shows a characteristic undershooting of gastric [K] like that found by MacMillan and Vane in cat plasma following histamine injection. The tissues of the GI tract may contribute more to these plasma K effects than the cells of any other organ. The mode of entry of such released K into the gastric lumen is still unknown, but transudation of interstitial fluid is a major possibility. [Grant A-2290, NIH, USPHS.]

2 *Physiology*

SITE OF GASTRIC SECRETORY STIMULATING ACTION OF AMINOGUANIDINE IN THE ALIMENTARY TRACT. K.W. Liepins (intr. by D. Nelson), Univ. Illinois College of Med., Chicago, Ill.

In a previous study it was found that aminoguanidine administered orally stimulated gastric secretion. In this study 2 Heidenhain and 2 total gastric pouch dogs were used. The aminoguanidine was always administered in a 0.4% concentration (base) in normal saline in a dose of 12 mg/kg. Application of the aminoguanidine solution to the Heidenhain pouch (14 tests) for 1 hour had no effect. Application of a 2.18% solution (8 tests) had no effect. Injection of the 12 mg/kg dose subcutaneously at one injection produced 26 and 43 mg HCl, respectively, in the two total pouch dogs. Application of the dose to the total gastric pouch for 1 hour produced 77 and 118 mg HCl, respectively. Introduction of the dose by tube into the intestine of the total pouch dogs produced 446 and 434 mg, respectively. The saline controls produced no acid.

3 *Physiology*

EFFECT OF HISTAMINE AND 1,2,4-TRIAZOLE ETHYLAMINE ON GASTRIC SECRETION OF HCl IS ADDITIVE. H.M. Janecek (intr. by A. Lane), Univ. Illinois College of Med., Chicago, Ill.

It has been found that 1,2,4-triazole 3 (beta ethylamino) , which is the triazole analogue of histamine (1,3-imidazole 4(beta ethylamino), stimulates the gastric secretion of HCl. Alpin and Lin reported that this triazole acts in an additive manner with histamine on gastric secretion. This study was undertaken to check on their report, since such an observation has an important bearing on the nature of gastrin. Two Heidenhain pouch dogs were used. They were given on different days 0.5 mg and 0.25 mg doses of histamine di-HCl and 0.7 mg and 0.35 mg doses of the triazole di-HCl. Then they were given on different days a dose of 0.25 mg of histamine di-HCl and 0.35 mg of the triazole di-HCl mixed together. Ten tests were done on each of the doses of the drugs. The data show that the two drugs act additively. Since they are so much alike chemically, an additive action might have been anticipated.

4 *Physiology*

INTERPRETATION OF EFFECTS OF AMINOGUANIDINE AND 1,2,4-TRIAZOLE ETHYLAMINE ON GASTRIC SECRETION IN LIGHT OF THE GASTRIN THEORY. A. C. Ivy, Univ. Illinois College of Med., Chicago, Ill.

The information on the effects of aminoguanidine and 1,2,4-triazole ethylamine may or may not have a direct bearing on the gastrin theory. This information provokes intriguing ideas regarding the pharmacology of the gastric HCl secretory mechanism.

5 *Physiology*

PAPER ELECTROPHORETIC DETECTION OF LEAKAGE OF ALBUMIN INTO THE GASTRIC JUICE IN PROTEIN-LOSING GASTROPATHIES AND GASTRIC CANCER. George B. Jerzy Glass and Akira Ishimori*.(Gastroent. Research Lab.,Dept. of Med.,New York Medical College, NYC)

Protein-losing gastropathies have been diagnosed by measurement of I^{131}-labeled albumin or PVP, or by chemical assay of albumin in gastric juice. Their detection also became possible by paper electrophoresis of gastric juice. Albumin forms an additional peak in the anodic area of the electrophoregram, close to the mucoprotein M_2. It differs from it by a marked affinity for amidoblack 10B and SF light green stains and by a low carbohydrate content when stained with PAS. We have detected passage of appreciable amounts of serum albumin into the gastric lumen in 26 of over 400 gastric juices studied by this method. Small amounts of albumin (3-10% w/w of non-dialyzable solids) were present in 7 juices of patients with histamine-fast anacidity and atrophic gastritis. We found them also in 6 other juices when no acid and pepsin were secreted; these juices at other times contained acid and pepsin but no albumin - probably digested by pepsin. By the same method we detected a massive leakage of albumin into the gastric juice (15-40% w/w of non-dialyzable solids) in 3 cases of Menetrier's disease, in 2 - of advanced gastric atrophy, and in 8 cases of gastric cancer - all associated with histamine-fast anacidity. (Supported by a grant from the American Cancer Society).

XUM

6 **Physiology**

EFFECT OF HISTAMINE METABOLITES AND HISTAMINASE INHIBITOR ON GASTRIC SECRETION IN DOGS. R. S. Alphin* and T. M. Lin. Lilly Research Laboratories, Indianapolis, Indiana.

Histamine (A) and its metabolites, N-methyl (B), N-dimethyl (C), Acetyl (D), 1-methyl (E) and 3-methyl-histamine (F) were tested for their action on gastric secretion, blood pressure and gut motility. A, B, C, and D all stimulated gastric secretion, whereas E and F were ineffective. N-Methyl and N-dimethylhistamine were sometimes even more potent than histamine itself on gastric secretion. Manometric determinations showed that A was the only substance susceptible to the action of histaminase in vitro. Simultaneous administration of histamine and a subthreshold dose of a histaminase inhibitor, aminoguanidine (AMG), augmented the action of histamine whereas the effect of the other metabolites of histamine, which are not substrates of histaminase, was not augmented by AMG. It is hypothesized that histamine and the histamine metabolites act on the same receptors. Histamine is physiologically involved in "triggering" gastric secretion and histaminase plays a role in the regulation of histamine-stimulated gastric secretion.

7 **Physiology**

TRANSPORT OF I^{131} IN DIFFERENT AREAS OF THE DOG STOMACH. M. H. F. Friedman, L. W. Rosen,* Joan Franco,* and S. Kramer.* Depts. of Physiology and Radiology, Jefferson Med. Coll., Phila., Pa.

Both acute experiments on anesthetized dogs and experiments on chronic animals equipped with isolated pouches of different stomach areas were carried out. Gastric juice was collected separately from the isolated gastric pouches under basal conditions and under the influence of various stimuli: histamine, mecholyl, insulin, electrical stimulation of the cervical vagus and sham feeding. The sodium salt of I^{131} was administered either intravenously or intramuscularly. Chloride sodium, acid, mucus, and pepsin concentrations were determined. The greatest concentrations of iodide occured under stimuli which evoked mucus secretion from either the isolated fundus or isolated antrum. Total output of iodide was independent of parietal cell activity. It was concluded that iodide in juice from the gastric corpus and fundus was derived only to a relatively small extent from parietal sources. (Supported by PHS grant No. 4508C2).

8 **Physiology**

SECRETORY STUDIES ON ISOLATED GASTRIC POUCHES IN MONKEYS. Cloyde L. Fausnaugh and Lloyd M. Nyhus (intr. by D.D. Feller.) Dept. of Surgery, Univ. of Washington School of Med., Seattle, Wash.

In order to further study gastric secretory physiology in the primate, vagally-innervated and vagally -denervated, isolated, parietal cell pouches have been developed for acute and chronic secretory studies in monkeys. Two monkeys with vagally-innervated pouches were studied. Twenty-four hour pouch collections showed a mean of 8.1 mEq. of free HCl in one animal and 8.4 mEq. in the other. Secretory curves for free HCl utilizing insulin hypoglycemia showed a characteristic sharp marked rise within one hour and a moderate rise in two to three hours. Initial depression the first one-half hour was usually, but not invariably noted. One animal with a vagally-denervated pouch was studied. Twenty-four hour secretion of free HCl averaged 1.31 mEq. No free HCl was present in the fasting state. Insulin hypoglycemia produced a depression in volume and total HCl, commencing the first hour and lasting up to five hours. Histamine (.06 mg. histamine base/Kg. body weight) produced a marked rise in volume and free HCl in this animal. Insulin hypoglycemia produced a significant drop (up to 75%) in both volume and free HCl after histamine. (Supported by Grant No. A-2187, National Institute of Health.)

9 **Pharmacology**

EFFECT OF A POTENTIALLY ULCEROGENIC "STRESS" ON GASTRIC SECRETION IN THE RAT. Rene Menguy (intr. by H.A. Shoemaker). VA Hosp. of Okla. City and Univ. of Okla. Med. Center.

Considerable uncertainty exists regarding the effects of "stress" on gastric secretory activity. It is generally thought that stress leads to gastric hypersecretion and ulceration. Studies were carried out on 100 albino rats weighing approximately 200 gm. Without any previous preparation 40 rats were stressed by restraint in a fine mesh wire "corset" for 20 hours. 20 random controls were placed in individual cages. At the end of 20 hours hemorrhagic ulcerations were present in the gastric glandular mucosa of all of the stressed animals. Stomachs of the control rats were normal. In another series of experiments pylorus ligation was performed on 40 rats previously fasted for 48 hours. Postoperatively 20 of the rats were returned to individual cages and 20 were placed in a restraining "corset" as described above. 6 hours after pylorus ligation the rats were killed and gastric contents studied for volume and free acidity. Almost complete suppression of gastric secretion was uniformly observed in the stressed group of rats. Therefore, the development of gastric ulcerations in the secretory mucosa of the rat during a non specific stressful situation is accompanied by a decrease in gastric secretory activity.

10 **Pharmacology**

EFFECT OF HISTAMINE ANALOGS AND HISTAMINASE INHIBITOR ON GASTRIC SECRETION. T. M. Lin, R. S. Alphin*, F. G. Henderson and K. K. Chen. Lilly Research Laboratories, Indianapolis, Indiana.

The gastric-stimulating and other histamine-like actions of 40 histamine analogs of the imidazole, pyrazole, triazole and tetrazole series were compared. The behavior of these analogs as substrates of histaminase was determined manometrically in vitro. The simultaneous administration of each analog and a histaminase inhibitor, aminoguanidine, was also studied. Results showed that 1) the action of those analogs which stimulated gastric secretion and also behaved as substrates of histaminase was augmented by aminoguanidine, 2) the action of those analogs which stimulated gastric secretion but did not behave as substrates of histaminase was not augmented by aminoguanidine, and 3) the action of those analogs which did not stimulate gastric secretion was also not influenced by simultaneous administration of aminoguanidine irrespective of their behavior toward histaminase. The structure-activity relationship of the histamine-like substances on gastric secretion will be discussed.

132. GENETICS

1 **Physiology**

STUDIES OF THE PHYSIOLOGICAL BASIS(ES) FOR DIFFERENCES IN SERUM ALKALINE PHOSPHATASE LEVEL OF TWO LINES OF WHITE LEGHORNS. F. H. Wilcox (intr. by R. M. Fraps.) Univ. of Md., College Park.

A line of White Leghorns has been developed by selective breeding in which the serum alkaline phosphatase level at six weeks of age is double that of a control line. This investigation has endeavored to elucidate the physiological mechanism(s) operating to cause this difference. The liver, kidney, duodenum, brain, and lung were analyzed for alkaline phosphatase of fresh tissue from both lines by a modification of the method of Bessey et al. (1946). The level in bone was determined after three days of storage at 2°C. The high line was found to have a significantly higher level in its kidney, but not in any of the other organs studied. In other experiments chicks from both lines were fasted one and two days; the level in the serum of the control line remained unchanged after fasting but was lowered somewhat in the high line after two days but not one day of fasting. When sera of the two lines were mixed in equal proportions, alkaline phosphatase activity was intermediate to that of the separate lines. The data obtained here have been taken to indicate that the genetic differences in the serum are due, at least in part, to a higher level in the kidney of the high line, and that a dietary influence or an inhibitor is not directly involved. (Supported by USPHS, Grant No. RG-6507)

2 *Biochemistry*

GENETIC CONTROL OF THE α- AND β-CHAINS OF HEMOGLOBIN. H. A. Itano and E. Robinson.* Natl. Insts. of Health, Bethesda, Md.

The technique of asymmetrical recombination reveals whether the α- or β-chain of an abnormal hemoglobin (Hb) carries its inherited defect (Nature 183:1799, 184:1468, 1959). Genes for Hb Hopkins-2 (Ho-2) and Hb S segregate independently, and double heterozygosity with respect to these genes and their normal alleles results in electrophoretic components with mobilities of Ho-2, A and S (Smith and Torbert, Bull. Johns Hopkins Hosp. 101:38, 1958). Recombination of mixtures of Ho-2, A and S resulted in increase of the A component with decrease of Ho-2 and S. Recombination of a mixture of Ho-2, A and C resulted in increase of A, formation of a new component between A and C, and decrease of Ho-2 and C. These changes indicate the presence of a defect in the α-chain of Ho-2. The intermediate electrophoretic component of the double heterozygote, which has the mobility of A, was isolated chromatographically and recombined. The observed effects are consistent with the presence in this component of both A and a molecule composed of the abnormal α- and β-chains, respectively, of Ho-2 and S. Our results, together with the familial data of Smith and Torbert, signify that nonallelic loci determine the structural defects of the α-chain of Ho-2 and the β-chain of S and that double heterozygosity at these loci results in four molecular species of adult hemoglobin.

133. GENETICS–Metabolic Errors

1 *Biochemistry*

PHENYLALANINE TURNOVER IN PHENYLKETONURIA. H.-D. Grülmer, H. Koblet, and C. Woodard (intr. by E. H. Frieden). The Boston Dispensary, Boston, Mass., and Pineland Hospital, Pownal, Maine

In phenylpyruvic oligophrenia (Fölling's Disease) phenylalanine (Phe) is mainly stored, incorporated into proteins, or excreted. 3 patients with this disease were maintained on a diet low in Phe with resulting blood levels of 2.3, 7, and 17 mg per 100ml of plasma. Phenylpyruvic acid was not demonstrable in the urines. Universally labeled L-Phe-C^{14} was injected intravenously and the disappearance of the labeled Phe from the protein free plasma filtrates was measured for 3.5 hrs. The curves obtained were composed of at least two components with different slopes, following first order reactions. Calculations based upon the slopes of the first component in these three patients indicate that a) the free Phe space is independent of plasma concentration of Phe and b) the absolute turnover rate decreases slightly with increasing pool size. The ratio of Phe space to antipyrine space was observed to be 1.53, 0.86 and 1.26. The latter finding indicates that Phe is probably concentrated intracellularly.

(Supported by USPHS Grant M-2729)

2 *Biochemistry*

IMMUNOLOGICAL STUDY OF HUMAN MUSCLE LACKING PHOSPHORYLASE. Phillips W. Robbins. The Rockefeller Institute, New York.

Enzymatic studies have previously been reported on a biopsy obtained from a patient lacking muscle phosphorylase (R.Schmid, P. W. Robbins, and R. R. Traut, Proc. Nat'l. Acad. Sci., 45, 1236, 1959). These experiments confirmed the lack of phosphorylase and showed the presence of UDPG-glycogen synthetase. The results provided strong evidence that glycogen is synthesized by way of UDPG and degraded by way of phosphorylase. A further point of interest in connection with this patient was the question of whether an inactive phosphorylase-like protein might be present in the muscle. This question was investigated immunologically. Antibodies to phosphorylase were prepared by injection of recrystallized rabbit muscle phosphorylase a or b into roosters. Both enzymatic inhibition and precipitation by the antibodies were observed. The antibodies were found to cross-react well with extracts from other mammalian muscle, including normal human muscle. No precipitation occurred with extracts from the muscle tissue of the patient lacking phosphorylase. The negative response indicates a level of phosphorylase antigen below 2% of that found in normal human muscle. It is concluded that phosphorylase-like protein is either completely lacking from the muscle or that it is modified so extensively that it is inactive both enzymatically and antigenically.

3 *Biochemistry*

BIOCHEMICAL BASIS OF HEREDITARY GALACTOSE SENSITIVITY. George J. Spyrides* and Herman M. Kalckar. McCollum-Pratt Inst., Johns Hopkins University, Baltimore, Maryland.

Unbalanced growth of bacteria due to a defect in galactose metabolism can arise in different ways. E. coli mutants defective in Gal-1-P uridyl transferase grown on glycerol ammonia mineral medium induce themselves upon addition of galactose into a state that can be described as "galactose induced bacteriostasis". The stasis is rapidly overcome by glucose. During the stasis Gal-1-PO_4 accumulates in amounts of 5 to 6 micromoles per gram wet weight. The accumulation continues during the restored growth brought about by glucose. Addition of Galactose-C^{14} to the static cells shows that radioactivity accumulates in the Gal-1-PO_4 fraction as well as in the insoluble complex polysaccharide fraction.

Another E. coli galactose negative mutant, C7M, undergoes lysis upon addition of galactose (Fukasawa and Nikaido). It has been found that this strain is defective UDPGal-4-Epimerase. In the presence of galactose during a brief static period preceding lysis a large increase in the amount of uridinediphospho galactose (UDPGal) is observed; almost 95% of the UDP-Hexose can be accounted for as UDPGal. Even after the addition of glucose which prevents lysis and restores growth the levels of UDPGal remain abnormally high.

4 *Biochemistry*

STABILIZATION AND INACTIVATION OF GLUCOSE-6-PHOSPHATE DEHYDROGENASE OF NORMAL AND MUTANT SUBJECTS. Paul A. Marks and Arieh Szeinberg*, Columbia Univ. Col. of Phys. & Surgs., New York.

In full hemolysates or stroma free hemolysates plus stroma, glucose-6-phosphate dehydrogenase (G6PD) of normals (N) is more stable than that of mutants (M), even when N stroma free hemolysate is diluted to the G6PD level of M. Experiments with mixtures of N and M hemolysates provide no evidence of a G6PD inhibitor or absence of an activator in M hemolysates. However, stroma (N or M) inactivates the N and M purified G6PD at similar rates. The stroma factor inactivating G6PD is heat labile and inhibited by TPN or nicotinamide. Stroma has TPNase activity. Purified preparations of N and M enzymes contain bound pyridine nucleotide which stabilizes them. The stabilities of N and M purified G6PD decrease with dilution and are enhanced by TPN or TPNH at 5×10^{-6}M, but not DPN. Purified G6PD of N and M do not differ in substrate affinities and other kinetic studies or electrophoretic mobility. Thus, the stability difference between N and M G6PD in hemolysates reflects an enzyme alteration or a factor modifying enzyme stability, which is not detected after purification of the enzymes.

5 *Biochemistry*

QUANTITATIVE STUDY OF URINARY MUCOPOLYSACCHARIDE EXCRETION IN GARGOYLISM. Melvin Fried and Thomas N. Campbell (intr. by H. J. Teas.) Univ. of Florida College of Med., Gainesville, Fla.

Gargoyles show increased urinary excretion of a mixture of mucopolysaccharides. In the present study, methods for the quantitative estimation of urinary mucopolysaccharide were developed and applied to a family with the sex-linked form of gargoylism. The possibility of detecting increased excretion of mucopolysaccharide by carriers of the trait as well as affected individuals was investigated. Three analytical methods were used. The first was a comparison of the reducing power of urine before and after acid hydrolysis, increase in reducing power being proportional to the mucopolysaccharide present. The second was a determination of the extent to which periodic acid was reduced by the urine, a quantity proportional to mucopolysaccharide content. The sensitivity of the method could be increased by prior HCl hydrolysis of the urine. The third technique was a modification of Lorincz and Dorfman's precipitation test in which the insoluble complex formed by mucopolysaccharides and acidified bovine serum albumin is measured turbidimetrically. A qualitative two-dimensional electrophoresis-chromatography "fingerprint" method was also developed. Parallel quantitative results from all three methods indicated that only clinically proven gargoyles, and not known carriers, excrete significantly high amounts of mucopolysaccharide.

XUM

Biochemistry

6

ERYTHROCYTE DIAPHORASE: THE ENZYMATIC DEFECT OF HEREDITARY METHEMOGLOBINEMIA. E. M. Scott, Arctic Health Research Center, Anchorage, Alaska.

A diaphorase which is specific for DPNH but which will reduce 2,6-dichlorophenolindophenol, cytochrome-c and methemoglobin is lacking in red cells of persons with hereditary methemoglobinemia (Biochem Biophys. Acta 34,584, 1959). By measuring rate of dye reduction, the amount of enzyme was determined in 250 Ingalik Indians, in whom methemoglobinemia is relatively frequent. Parents of methemoglobinemic children had less diaphorase than did normal persons, and a bimodal frequency distribution of enzymatic activities was obtained in this population. The values were consistent with inheritance of methemoglobinemia as a recessive trait. The erythrocyte diaphorase of normal cells has been purified 3000 times and its properties determined. Kinetic evidence indicates that DPNH quickly reduces the enzyme which in turn reduces the other substrate at a much slower rate.

Biochemistry

7

DEPENDENCE OF URINARY INDOLE EXCRETION IN HARTNUP DISEASE UPON GUT FLORA. K. N. F. Shaw, D. Redlich*, S. W. Wright* and J. B. Jepson*. Calif. Inst. of Technology, Pasadena; Univ. Coll. Hosp. Med. School, and Middlesex Hosp. Med. School, London.

The reported high excretion of urinary indoles in Hartnup disease has been studied comparatively in an original Hartnup patient and a normal individual by means of paper chromatography. Neomycin and diet free of plant materials were used to curtail exogenous indoles. With this restriction, metabolites excreted by the Hartnup patient after oral indolepyruvic acid or L-tryptophan were qualitatively normal but amounts were subnormal; excretion of tryptophan itself was continuous. Urinary indoleacetic, indolelactic and xanthurenic acids increased in the normal subject after oral tryptophan. When tryptophan was given to the Hartnup patient without restriction, xanthurenic acid was absent and excretion of indoleacetic and indolelactic acids, and indican, indoleacetylglutamine, indoleacetylglucuronide and indoleacrylylglycine was abnormally high, and prolonged or delayed. Levels of tryptophan and indican, but not other indoles, were above normal in random Hartnup urines. The results indicate that intestinal microorganisms are the source of abnormal indoles in Hartnup disease. Impaired intestinal absorption of L-tryptophan and possible toxic effects of abnormal metabolites also are suggested as features which may be pertinent to the disorder.

Pharmacology

THE EFFECT OF RESERPINE ON URINARY TRYPTAMINE AND INDOLE-3 ACETIC ACID EXCRETION IN MENTAL DEFICIENCY, SCHIZOPHRENIA AND PHENYLPYRUVIC OLIGOPHRENIA. G. W. G. Brune* and H. E. Himwich, Galesburg State Research Hospital, Galesburg, Illinois.

The urinary excretion of tryptamine and indole-3-acetic acid, was determined according to Sjoerdsma et al., in 4 mental defectives, 11 schizophrenics, 2 phenylpyruvics. The daily urinary excretions were determined during 3 periods composed of 20 days each for control, experimental and second control. The patients received a constant diet and the 24-hour urinary specimens were either worked up immediately or frozen. The urinary tryptamine calculated as tryptamine/day and tryptamine/g creatinine/day was within normal limits during the first control period. The results did not follow any single pattern. During reserpine administration the mental defectives exhibited a decrease of 20 to 50%. Two schizophrenics showed a decrease of 20%; no change in 4, and an increase of 20 to 30% in another 4, and 100% in one. Some of the schizophrenics showed a significant increase of urinary tryptamine during the first day of reserpine medication. A similar arrangement of results was noted for indole-3-acetic acid. In the oligophrenics the total indole-3-acetic acid was elevated throughout.

134. GENITOURINARY TRACT

Pharmacology

1

EFFECTS OF SOME LOCAL ANAESTHETICS, GANGLION- AND NMJ-BLOCKERS ON THE ELECTRICALLY STIMULATED ISOLATED DOG URETER. Harold H. Borgstedt (intr. by H. C. Hodge.) Dept. of Pharmacology, University of Rochester, Rochester, N. Y.

The drug effects were studied on the perfused isolated dog ureter in a system which permitted electrical stimulation with DC rectangular impulses or sinusoidal AC and recording of resultant longitudinal contractions. The results gained with either method of stimulation were essentially the same. When added to the bath fluid, some representative drugs of the following classes: local anaesthetics (procaine, tetracaine, lidocaine), ganglion-blockers (tetraethylammonium HCl, hexamethonium HBr), and NMJ-blockers (d-tubo-curarine Cl, succinylcholine Cl, decamethonium HBr) inhibited ureteral contractility in very high concentrations only and then inconsistently. It can therefore be assumed that the smooth muscle cells of the ureter are stimulated directly and that drug effects observed in this system are also directly muscular. This invest. was supp. by PHS grant # B1149 from the National Institutes of Health, PHS.

Pharmacology

2

THE PERISTALTIC REFLEX OF THE ISOLATED DOG URETER. MARSHALL E. ATWELL AND HAROLD H. BORGSTEDT. (INTR. BY H. C. HODGE.) DEPT. OF PHARMACOLOGY, UNIVERSITY OF ROCHESTER, ROCHESTER, NEW YORK.

SINCE IN VIVO THE URETER HAS A FUNCTION SOMEWHAT SIMILAR TO THE INTESTINE THIS STUDY WAS DONE TO ELUCIDATE ONE SIMILAR MECHANISM - THE "PERISTALTIC REFLEX". LONGITUDINAL CONTRACTIONS OF URETERAL SEGMENTS WERE RECORDED DURING A CONTROLLED GRADUAL ELEVATION OF INTRALUMINAL PRESSURE. THE FIRST CONTRACTION GENERALLY OCCURRED AT A PRESSURE OF 2 TO 6 CM OF H_2O, IN ONE CASE AT A PRESSURE AS HIGH AS 16 CM. IN MOST INSTANCES IT WAS NECESSARY TO ADD HISTAMINE IN CONC. OF 0.2 TO 0.6×10^{-5} M TO THE BATH FLUID TO BE ABLE TO ELICIT THE PERISTALTIC REFLEX. THOUGH DEVOID OF A NERVOUS PLEXUS, THE URETER ACHIEVES FLUID TRANSPORT BY A PERISTALTIC MECHANISM SIMILAR TO THAT OF THE INTESTINE. HISTAMINE SENSITIZES THE SMOOTH MUSCLE CELLS FOR THIS FUNCTION. (THIS INVEST. WAS SUPPORTED BY PHS GRANT NO. B1149 FROM THE NATIONAL INSTITUTES OF HEALTH, PHS. M.E.A. SUPP. BY TOBACCO IND. RES. COMM. STUDENT FELLOWSHIP.)

Pathology

3

EXPERIMENTALLY INDUCED KWASHIORKOR IN RABBITS. R.W. Volk and S.S. Lazarus. Isaac Albert Res. Inst. of the Jewish Chronic Disease Hospital, Bklyn., Department of Path., Albert Einstein College of Medicine, Bronx, N.Y.

A study was conducted on the production of experimental Kwashiorkor in rabbits utilizing a diet which simulated that of poor class Jamaicans. This diet consisted of 20% white potatoes, 22.5% green bananas, 30% corn meal, 20% brown sugar and 7.5% powdered whole milk (Klim). It contained approximately 5.8% protein, 2.2% fat and 73.9% carbohydrate. The animals showed a marked weight loss which ranged from 20 to 50% of initial body weight as well as marked loss of hair. From the 9th week of treatment on there were frequently severe pancreatic changes. The acinar cells were atrophic and shrunken with reduction of zymogen granules and frequently groups of shrunken necrobiotic acini were present. In addition, in many instances, the acinar cells showed cytoplasmic vacuolization. The ductular epithelium showed no definite proliferative activity. In some instances areas of peripancreatic fat necrosis and focal pancreatitis were noticeable. The liver showed varying degrees of fatty metamorphosis with eventual development of cirrhosis. The pancreatic alterations are considered to be due either to protein deficiency or deficiency of a specific amino acid. The hepatic changes preceded or developed parallel with those in the pancreas and are considered to be a primary effect of the diet and not secondary to impaired pancreatic function.

136. IMMUNE RESPONSE

1 **Physiology**

EVIDENCE FOR A LETHAL AUTO-IMMUNE RESPONSE IN SEVERELY BURNED RATS. Stephen Atherton*, Nancy Merrill*, and Miles D. McCarthy. Department of Zoology, Pomona College, Claremont, California.

Among male Wistar rats which received a standardized back burn of 32% of the body surface in water at 90°C. for 35 seconds there was a high incidence of positive direct Coombs tests in the period 48-96 hours post-burn. All animals whose direct Coombs tests showed +++ or ++++ agglutination died within 24 hours of the test. All rats demonstrated negative direct Coombs tests before the burn. Viable cell suspensions prepared from the lymph nodes and spleens of those animals dying with a positive direct Coombs test were injected intraperitoneally into rats with negative direct Coombs tests. The recipients developed a positive direct Coombs test and died. Control rats receiving cell suspensions from Coombs negative rats failed to develop a positive direct Coombs test. This evidence indicates that an auto-immune mechanism is responsible for the positive direct Coombs test and that this is strongly correlated with mortality. (Supported by USPHS Contract A-1690 and by grants from the California Heart Association and the NSF.)

2 **Physiology**

"TOXIN-ANTITOXIN" PHENOMENA IN INJURED ANIMALS AND MAN (THERMAL, RADIATION, AND PHYSICAL INJURY). Sol Roy Rosenthal, Wilma A. Spurrier* and Alan B. Goodman*. University of Illinois College of Medicine, Chicago, Illinois.

By serologic methods it was found that shortly after injury a "toxin(s)" appears in the blood of mice, rats, and humans. With healing of the lesions, an "antitoxin(s)" appears. In scalded (70°C to 96°C) or irradiated (Strontium 90; 1000 to 4000 rep) animals and burned (by various means) or injured (fractures) human subjects, "cytotoxins" (RBC of acutely injured plus sera of same and guinea pig complement) and precipitogens (sera of acutely injured plus sera of healed injured) appear in the blood shortly after injury. With healing of the lesions, precipitins (to sera of acutely injured hosts) and hemolysins (to RBC of acutely injured hosts plus guinea pig complement) appear in the blood. Tissue culture studies to be reported elsewhere support this "toxin-antitoxin" phenomenon. In vivo studies indicated that the blood or plasma of healed burned human subjects when transfused into acutely burned children markedly improved their clinical condition as indicated by decrease in pain, edema, lowering of temperature and urinary albumin. (Aided by grants from the Office of Naval Research (NR 114 161) and the Atomic Energy Commission (AT(11-1)-706)

3 **Physiology**

COLD AGGLUTININS IN NORMAL SUBJECTS IN RELATION TO PREVIOUS ILLNESS, BLOOD GROUP, AND AGGLUTININS FOR TRYPSINATED RBC. W. W. Christenson (intr. by Harold G. Wolff) New York Hospital-Cornell Medical Center, New York.

157 men (ages 26-32) were carefully examined and interviewed to determine present health and past illness. None had evidence of blood dyscrasia. Their sera were studied for titer of anti-A, anti-B, and of agglutinins for normal and trypsinated RBC (TRBC) after 2 hours at 4° C, room temperature (23-29° C) and 37° C. At 4° C all sera agglutinated the TRBC (titer 16-128); 88 agglutinated the normal RBC (titer 1-32). At room temperature 50 sera agglutinated the TRBC (titer 2-64), usually with a prozone; of these, 13 weakly agglutinated the TRBC at 37° C, constantly with a prozone. There was no correlation between titer of agglutinins for normal or TRBC at any temperature with major blood group, titer of anti-A or anti-B, or previous illness experience. Agglutination of TRBC at higher temperatures appeared to be due to incomplete reversal of "normal autoagglutinin," reversal in lower dilutions producing the prozone. Further studies suggest that the higher titers of "cold agglutinins" for TRBC are due to increased avidity of "normal autoagglutinin" and failure of its reversal at 4° C. It is concluded that titers of cold agglutinin or agglutinins for TRBC do not reflect rates of previous illness in "healthy" subjects. (Supported by Dept. of Army Grant #DA-49-007-MD-524.)

4 **Biochemistry**

FRACTIONATION OF THE ALLERGENIC PROTEINS OF CASTOR SEED. Laurence L. Layton*, Floyd DeEds and Lloyd Moss*. Western Regional Research Laboratory1/, Albany, Calif., and Stanford Research Institute, Menlo Park, Calif.

Castor bean seed protein has long been recognized as a potent allergen. Spies and coworkers (J.A.C.S., 1943, 65, 1720) have prepared an allergenic material which has been designated as the principal allergen and coded CB-1A. Soluble castor bean protein and a sample of castor bean allergen CB-1A have both been chromatographed on an anion exchange column. By pH gradient elution, the soluble castor bean protein was separated into twelve major protein species; the allergen preparation CB-1A was found to contain the same twelve proteins in somewhat altered quantitative distribution. Guinea pigs sensitized to the allergen CB-1A were sensitive to each of the twelve component protein fractions. Animals sensitized to either the pollen or the female blossoms of castor were also sensitive to the castor bean seed protein. It would appear that allergy to castor may in reality be sensitivity to one or more members of a complex mixture of at least twelve distinct protein species. Dust containing fragments of pollen grains or female blossoms will sensitize guinea pigs to the seed proteins.

1/. Western Utilization Research and Development Division, Agricultural Research Service, U.S. Dept. of Agriculture.

5 **Pharmacology**

CLINICAL EVALUATION OF A NEW ANTIHISTAMINE (CYPROHEPTADINE). Tibor Bodi, Peter E. Siegler, Marvin Gershenfeld, Elizabeth B. Brown and John H. Nodine (intr. by John H. Moyer.) Hahnemann Med. Coll. and Hosp., Philadelphia, Pa.

Cyproheptadine, 1-methyl-4-(5-dibenzo-[a,e]-cycloheptatrienylidene)-piperidine hydrochloride monohydrate, possesses both antiserotonin and antihistaminic activity in animals. It protects against experimental anaphylaxis. Cyproheptadine was evaluated in 50 ambulatory patients, suffering from various allergic disorders. The average duration of therapy was 41 days, and the doses ranged from 2 to 20 mg. daily. Approximately three-fourths of the patients showed definite improvement and most of these had a clinically satisfactory response. The best therapeutic dose varied between 15 and 20 mg./day. No tolerance developed to the drug, and side effects, which were few and transitory, included drowsiness and dizziness. One questionable case of mild reversible leukopenia was observed. The drug was most effective in allergic skin disorders and vasomotor rhinitis. It relieved pruritus associated with allergic dermatitis, urticaria and neurodermatitis. The drug was not generally effective in patients with bronchial asthma. It was effective in three cases of tension headache and lowered histamine-like amine levels in the blood of 5 of 6 patients studied.

6 **Immunology**

AUTOIMMUNE NEPHROSIS IN RATS. W. Heymann, D. B. Hackel and J. L. P. Hunter (intr. by J. H. Dingle.) Western Reserve University, Cleveland, Ohio.

Nephrotic renal disease has been produced in 60 rats by 6-10 intraperitoneal injections of Freund's complete adjuvants and rat kidney suspensions given every 2 weeks. This was also noted in 8 of 10 rats in whom preceding unilateral nephrectomy provided their own kidney material for injection. Rat liver suspensions led less regularly to a less severe, but identical renal lesion. Muscle and lung tissue failed to induce renal disease. Renal cortex was more active than renal medulla. ACTH, cortisone and HN_2 effectively inhibit the development of this experimental disease. Using Ouchterlony's technique, single bands of precipitation were noted when sera of experimental rats were tested with rat kidney antigen. These were less frequently obtained with liver antigen, and never observed with rat muscle or lung suspensions. The bands obtained with liver and kidney gave a reaction of identity. When kidney antigen was obtained from rabbits or guinea pigs, cross-reactions were observed. Hemagglutination titres were rarely obtained but complement decreased regularly. (Supported by a grant from the N.I.H.)

1 *Immunology*

EFFECT OF PROLONGED IMMUNIZATION ON IN VITRO AND IN VIVO PROPERTIES OF RABBIT ANTIMOUSE ERYTHROCITE SERUM. (abstract) Israel Davidsohn and Changling Lee*. Depts. of Pathology, Chicago Medical School and Mount Sinai Hospital, Chicago, Ill.

In studies on heteroimmune hemolytic anemia in mice we observed that after three courses of 4 weekly injections of mouse erythrocytes additional injections produced no significant changes in agglutinin and hemolysin titers but the resulting anemia was decreased significantly. In order to check these observations 3 rabbits were given 7 courses. They were bled 7 days after the 4th injection of each course. All serums were tested simultaneously after the 7th course: 1. The highest agglutinin and hemolysin titers were reached at the end of the third or fourth course, and remained approximately the same. 2. The anemia reached its peak at the end of the 3rd or 4th course and afterwards continued to drop significantly. 3. The dissociation of in vitro and in vivo activity of rabbit antimouse erythrocyte serum is in accord with our results obtained in the study of electrophoretic fractions of these serums. 4. The antibody titers which are frequently used as indicators of the degree of immunization are not necessarily applicable to the in vivo effect. (Supported in part by grant A-77 of the National Institutes of Health, United States Public Health Service and by the Mount Sinai Medical Research Foundation).

8 *Immunology*

IMMUNOLOGICAL TOLERANCE AND VIRUS INFECTION WITH LYMPHOCYTIC CHORIOMENINGITIS IN MICE. John E. Hotchin, Heribert Weigand and Lois M. Benson (intr. by Sophia M. Cohen.) Div. Laboratories and Research, N.Y. State Dept. Health, Albany.

Mice were challenged intracerebrally with LCM virus at the following ages: 2, 6, and 12 hrs., 1, 2, 3, 4, 5, 6, and 7 days, after which weight gain and mortality of the animals were followed. Mortality was 80% in the 2-hr. group but dropped to 30% in the 6- and 12-hr. groups, rising to 60% at 24 hrs. and 100% in the 4-7 day groups. Concomitantly, marked depression of growth rate was noted, producing "runt" mice. The growth rate of survivors suddenly returned to the normal rapid value; these mice contained high titers of virus in brain and blood for many months although appearing to be perfectly normal. Virus-carrying mice (designated "vital") produced no complement-fixing or neutralizing antibody to the virus unlike LCM immune adult mice which had suppressed LCM infection. The "vital" animals showed no histological abnormality. Fluorescent antibody technic indicated that most of the cells throughout the "vital" mice contained LCM antigen. This work indicates that immunological tolerance to a virus can be acquired by mice exposed to it at a very early age, resulting in prolonged harmless virus multiplication for a large part of their normal life span.

9 *Immunology*

STUDIES OF SERUM COMPONENTS RELATED TO IMMUNITY IN ANIMALS CHEMICALLY PROTECTED AGAINST X-IRRADIATION. W. A. Hook*, L. H. Muschel and G. Edsall. Dept. of Serology, Div. of Comm. Dis., Walter Reed Army Institute of Research, Wash., D.C.

Guinea pigs were pre-protected against 400 r doses of total body x-irradiation by intraperitoneal inoculation of diphenylamine, p-hydroxydiphenyl, p-aminopropiophenone, cysteine, and mercaptoethylamine (Jacobus, D. P., Fed. Proc. 18:74, 1959, and personal communication). Sera were assayed for lysozyme, properdin, T2 bacteriophage neutralization, total hemolytic complement (C'), and normal bactericidal antibody against Shigella dysenteriae. Protection consistantly decreased the decline of lysozyme levels observed following irradiation. Properdin titers usually declined by the 14th day or later after irradiation, but the effects of protection were variable. Irradiation or protection did not greatly alter bacteriophage neutralizing activity of these sera. C' titers fell to below normal approximately 3 days after irradiation and protected animals experienced a similar decline. A slight protective effect was observed against the drop in bactericidal antibody observed 3 to 7 days after irradiation. Generally when losses of lysozyme and bactericidal antibody occurred as a result of irradiation, chemical protection afforded a slight defense against these declines.

10 *Immunology*

PHAGOCYTOSIS OF INERT BENTONITE PARTICLES FOLLOWING THE ADSORPTION OF VARIOUS ANTIGENS. Elizabeth V. Potter, Northwestern Univ., Chicago, Illinois. (intr. by G.H.Stollerman.)

Surface antigens and their reaction with opsonizing plasma factors were studied in an effort to better understand the processes of virulence and immunity. Bentonite particles were shown to be suitable for this purpose because they were of proper size and shape, poorly phagocytized by human leucocytes when in appropriate dilution, bound to proteins firmly, and stained adequately. Phagocytic indices in human blood were increased from 5% to 100% by adsorption to the particles of 1) pooled human gamma globulin (Cohn Fr. II) or 2) bacterial antigens and subsequent addition of homologous antibody. Similar treatment of particles with Cohn Fr. V caused no increase in phagocytosis. Adsorption of purified streptococcal M protein caused moderately increased phagocytosis which was not blocked by the further addition of streptococcal hyaluronic acid. Hyaluronic acid alone did not affect phagocytosis. Phagocytic activity of all systems studied was markedly reduced in heated plasma and further reduced when buffers were substituted for plasma as the suspending medium. The affinity of different adsorbed substances for phagocytes varied markedly, however, even in the absence of plasma factors. Particles coated with gamma globulin were taken up most avidly.

11 *Immunology*

LYSOZYME CONTENT OF NORMAL RABBIT ALVEOLAR MACROPHAGES. Quentin N. Myrvik, Eva Soto Leake* and Bruce L. Fariss*. Dept. of Microbiology, Univ. of Virginia School of Med., Charlottesville, Virginia.

Mononuclear type cells can be procured from the alveoli of normal rabbit lungs by introducing Hank's balanced salt solution into the trachea and washing the cells out. The cells resemble macrophages and are capable of phagocytizing particulate material. The cells obtained are essentially all mononuclear (less than 1 per cent polymorphonuclear cells). Cell free extracts were prepared by alternate freezing and thawing or by sonic oscillation. They contain large amounts of lysozyme (2,000 to 4,000 micrograms per ml of packed cells). Since approximately 5 to 10 million macrophages can be recovered from the normal rabbit lung in a high state of purity, it is probable that these cells are the "normal scavenger flora" of the lower respiratory tract. (Supported by a grant from USPHS E-1238-C2).

12 *Immunology*

QUALITATIVE ASPECTS OF C-REACTIVE PROTEIN. C. W. Fishel and N. A. Vedros (intr. by R. Thompson.) Dept. of Microbiol., Univ. of Colo. School of Med., Denver, Colorado.

Sera from hospitalized individuals were tested for the presence of C-reactive protein using commercially prepared antiserum (CRPA-Schieffelin). Positive specimens were fractionated by Cohn's method and with 6,9-diamino-2-ethoxy-acridine lactate (Rivanol). The reactive substance was localized in Fractions I+III-3 and III-1,2, and was precipitated by Rivanol. These data suggest a possible relationship between CRP and insoluble globulins. The agar diffusion method of Allison and Humphrey (Nature 183: 1590, 1959) indicated that CRP contained 3 antigenic components with diffusion coefficients (D_{20}) of 1.26, 2.36, and 3.54; approximate molecular weights of 2.0×10^6, 6.0 and 2.5×10^5. Electrophoretic separation of serum in agar followed by staining for protein, lipo- or mucoprotein, or for mucopolysaccharide, failed to show a significant increase of any component which could be correlated with the presence of CRP. The reactive substance was in the same immunoelectrophoretic position regardless of pH of the agar and buffer. (Supported by USPHS grant E-2653.)

13 *Immunology*

RATE OF DISAPPEARANCE OF Cr^{51} TAGGED ERYTHROCYTES IN RABBITS RENDERED UNRESPONSIVE TO ISO-ANTIGENS. Carl Cohen and Harry C. Gurney (intr. by M. C. Dodd) Battelle Memorial Institute, Columbus, Ohio.

Introduction of incompatible blood into newborn rabbits is capable of rendering them unresponsive to a later encounter with these same erythrocytic iso-antigens. Variation in the length of the period of treatment results in a variation in iso-antibody production ranging from a significant but not complete inhibition to complete inhibition. The results, based upon levels of circulating antibody, are being extended by the use of the rate of disappearance of transfused cells of the incompatible type tagged with Chromium-51. In animals shown by antibody titres to be completely unresponsive the rate of disappearance resembles that of the unimmunized control animals. In animals with a partial inhibition of antibody production, as shown to be a relatively low titre of iso-antibody, the rate of disappearance resembles that in animals which are typically immune to the red cell antigens. (Supported by Research Grant RG 5938 from the National Institutes of Health, United States Public Health Service.)

14 *Immunology*

RABBIT ANTIBODIES AGAINST γ-GLOBULINS RESEMBLING THE RHEUMATOID FACTOR. Felix Milgrom* and Ernest Witebsky, Univ. of Buffalo School of Med., Buffalo, N. Y.

One group of rabbits was injected with γ-globulin obtained by ammonium sulfate precipitation from the animal's own preimmune serum. A second group was injected with heat-killed β hemolytic streptococci. Both groups produced very similar antibodies of anti-γ-globulin specificity proven by immunoelectrophoresis and absorption. Reactions with γ-globulins of foreign species, especially human, were much stronger than reactions with γ-globulins of their own species. To produce positive complement fixation and gel precipitation with the antisera in question, the rabbit γ-globulin preparations had to be at least a thousand times more concentrated than human preparations. It was concluded that the antibody production in both groups was stimulated by those molecules of the animal's own γ-globulins which had undergone changes depriving them of their species specificity. In the first group the alterations were probably induced by chemical isolation; in the second group they might have resulted as a sequela to the immune response to streptococci. The similarities between the described rabbit antibody and the rheumatoid factor favor the assumption that the latter is an antibody to an individual's own altered γ-globulins. (Supported by a grant from The National Foundation.)

15 *Immunology*

CYTOCHEMICAL STUDY OF THE INTERACTION BETWEEN BACTERIA AND PROTEIN WITH HIGH ISOELECTRIC POINT. John K. Spitznagel (intr. by John H. Schwab.) Dept. Bact. and Immunology. School of Med. Univ. of N. C., Chapel Hill.

Participation of proteins with high isoelectric points in the non specific antimicrobial defenses of the mammalian host has been suspected but difficult to evaluate due to the lack of techniques for demonstrating their interaction with parasites in vivo. Alfert (Proc. Nat. Acad. Sc. 39:991-999, 1953) has shown that Fast Green selectively stains histones and protamines at pH 8. Eighteen hour broth cultures of B. anthracis and E. coli resuspended and incubated at 37°C for 30 minutes in .05M McIlvaine buffer pH 5.6 (10^8 organisms per ml) do not take this stain. Similar treatment but with histone (50γ/ml) or basic polypeptides added to the buffer produces bacteria which stain well. Bacterial staining and killing as tested with E. coli are roughly parallel. Other methods of killing E. coli did not cause the bacteria to stain. Stainability of the histone treated organisms was abolished by treating them for 20 minutes at 37°C with 1% trypsin. It appeared that the bacteria were stained with Fast Green as a result of having actively or passively taken up the histone. Experiments with bacterial infections in animals indicate that it will be possible to study host-parasite interactions involving proteins with high isoelectric points using Fast Green. (Supported by USPHS grant SF-155 and E-2430.

137. IMMUNE RESPONSE—Antibody Formation

1 *Physiology*

THE SPECIFICITY OF IMMUNOLOGICAL DEPRESSION. H. Timourian and A. M. Schechtman (intro. by Clara M. Szego.) Zoology Dept., University of California, Los Angeles.

Depression of antibody production by injecting antigen into fetal or newborn animals shows a certain degree of specificity. The aim of the present study was to determine whether antigenic determinants for antibody production play an equivalent role in immunological depression. Injection of bovine gamma globulin (BGG) into neonatal rabbits resulted in almost complete depression of homologous antibody production at maturity, and a 70-80% depression in the capacity to produce antibodies versus bovine serum albumin (BSA) and human gamma globulin (HGG). Normal rabbit anti-BGG cross-reacts with BSA and HGG in the order of 3-6% of the homologous reaction. Chickens showed a response distinctly different from that of rabbits. Newly hatched chickens injected with BGG showed a strong depression of antibody against BGG at maturity, but no significant reduction in antibody against BSA and HGG. Normal chicken anti-BGG showed a 10% cross-reaction with HGG but none with BSA. Hence, in both chicken and rabbit, the specificity of immunological depression does not correspond quantitatively to the specificity of cross-reacting antibodies. (Aided by Predoctoral Fellowship, U.S.P.H.S., CF-9044-C1.)

2 *Physiology*

INHIBITION OF ANTIBODY FORMATION BY HOMOLOGOUS GAMMA GLOBULIN Ignatius L. Trapani and Dan H. Campbell (intr. by John B. Loefer.) Div. of Chemistry and Chemical Engineering, California Institute of Technology, Pasadena, California.

A preliminary investigation was initiated in an attempt to learn if γ-globulin or antibody formation could be repressed by multiple S.C. injections of homologous γ-globulin in complete Freund's adjuvant. Five groups of 10 rabbits each were treated during a 4-week period as follows: Groups a & b, adjuvant only; Groups c & d, adjuvant + homologous γ-globulin; Group e, no pretreatment. A serum sample was obtained after a 1-week rest period, and the animals of Groups a, c & e given a series of I.V. injections of BSA during the next 5 weeks; rested 1 week; and then bled. Free-boundary electrophoresis did not show any significant differences in the distribution of the various serum components between the control and experimental groups after either of the series of injections. Circulating anti-BSA levels (mg Ab protein/ml serum ± stnd. dev.) for Groups a, c & e were 3.87 ± 0.76, 1.86 ± 0.51 and 4.49 ± 0.98, respectively. None of the sera gave detectable precipitation reactions with the homologous γ-globulin used for the first series of injections. The data at hand indicate that antibody formation may be suppressed by prior treatment with homologous γ-globulin. (Supported by the National Institutes of Health.)

3 *Physiology*

THE ROLE OF THE EOSINOPHIL AND PLASMA CELL IN ANTIBODY FORMATION.

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Immune inflammation produced by the reinjection of antigen is characterized by the accumulation of two types of cells: the eosinophil granulocyte and a basophilic cell referred to as a transitional or immature plasma cell. The eosinophil response occurs prior to the appearance of serum antibody, and the plasma cell response occurs simultaneously with the appearance of serum antibody. Evidence to be presented suggests that plasma cells are formed from macrophages which have engulfed both eosinophils and antigen. The presence of phagocytized eosinophils within macrophages appears to produce a stimulation of RNA and protein metabolism and a temporary inhibition of DNA metabolism. These experiments also suggest that sensitization of an animal to a specific antigen is associated with the eosinophil granulocyte and its precursors.

4 *Biochemistry*

ROLE OF DISULFIDE BONDS IN THE STRUCTURE OF RABBIT ANTIBODY. A. Nisonoff, F.C. Wissler* and D.L. Woernley*. Roswell Park Memorial Institute, Buffalo, N.Y.

Peptic digestion of rabbit antiovalbumin γ-globulin at pH 5 decreases the sed. rate to about 5S; the capacity to precipitate specifically is retained. Subsequent treatment with a disulfide-splitting reagent at pH 8 yields nonprecipitating fragments with blocking activity and s≅3.5. Cysteine, 2-mercaptoethylamine, thioglycolate and cyanide were effective. These fragments are evidently univalent; in their quantitative inhibitory effects, s value and chromatographic behavior on carboxymethylcellulose, they are nearly identical with univalent fragments produced by papain; also, the combining sites on nonprecipitating fragments of antihapten antibody formed in this way were found, by binding measurements, to be essentially intact. Since S-S splitting reagents are used, as activators, with papain, hydrolysis by that enzyme may also occur in two stages. Our results, together with those of Porter, suggest that the antibody is composed of two nearly identical univalent subunits, linked by S-S bond(s), and an inactive fragment readily removed by proteolysis; it is suggested that biosynthesis may involve combination of these univalent units through oxidation of SH groups. Human γ-globulin is similarly broken down to s≅3.5 in two stages, by peptic digestion and reduction.

5 *Pathology*

GAMMA GLOBULIN LEVELS IN IMMUNIZED X-IRRADIATED RABBITS. Marvin B. Rittenberg* and Eric L. Nelson. Dept. of Bact., Univ. of Calif., Los Angeles, California.

Adequate doses of radiation prevent antibody formation in immunized animals. Studies on the mechanism of this inhibition previously were not concerned with measuring gamma globulin levels. Antibody formation in immunized animals is normally associated with increased gamma globulin, conversely there should be no increase in gamma globulin when antibody formation is blocked by radiation. Rabbits were exposed to 400r whole-body x-radiation and immunized intravenously 24 hr later with approximately 10 mg of bovine serum albumin (BSA) incorporated into hemoglobin particles (J. Exp. Med. 107:755, 1958). Anti-BSA antibodies were detected by the ammonium sulfate technique of Farr (J. Inf. Dis. 103:239, 1958) and by the disappearance of circulating I^{131}-BSA administered with the antigen (J. Immunol. 67:243, 1951). No antibody was found in serum from irradiated rabbits. Serums collected daily from all rabbits were analyzed by paper electrophoresis. Radiation did not appear to affect gamma globulin levels. Results will be discussed in terms of aberrant protein (gamma globulin) synthesis associated with immunization of x-irradiated rabbits. (Supported by a grant E-2298 from the National Institute of Allergy and Infectious Disease.)

6 *Immunology*

ENHANCING, ADDITIVE AND ANTAGONISTIC EFFECTS OF COMBINATIONS OF SALMONELLA OR PROTEUS IMMUNE SERA IN AFFORDING PASSIVE MOUSE PROTECTION. R. Lieberman and N. Mantel (intr. by D. J. Davis). Natl. Insts. of Health, Bethesda, Md.

Results are presented of passive mouse protection tests employing a variety of combinations of salmonella or proteus immune sera. Both native and absorbed antisera were used and these were homologous and/or heterologous to the challenge employed. The combinations tested included instances where neither one, only one, or both of the antisera tested, were individually protective for the challenge used. Challenges included S. enteriditis, S. typhimurium, P. vulgaris and P. mirabilis. Various criteria were used in assessing combined antisera activity. Simple additivity was observed for combinations of homologous antisera, whereas superadditivity was noted for combinations of an homologous antiserum with an effective heterologous antiserum. Zonal antagonism was seen whenever the test combinations included an ineffective heterologous antiserum. The problem of simultaneous protection against two challenges was also investigated. Clinical implications and implications with respect to theories of antibody synthesis relative to the observed phenomenon of enhancement and antagonism between antisera are discussed.

7 *Immunology*

EFFECT OF A RETICULOENDOTHELIAL SYSTEM (RES) STIMULANT ON ANTIBODY PRODUCTION. Daniel A. Boroff and James R. Reilly*. New England Institute for Medical Research, Ridgefield, Conn. and Conservation Department State of New York, Albany.

It has been shown that a certain RES stimulant, Restim, obtained from yeast cell walls stimulates the formation of hemolysin in rats injected with rabbit erythrocytes (Cutler, J. Immunol., in print). In order to determine whether Restim will also aid in antibody production when a soluble antigen like the toxoid of C. botulinum is employed, mice were injected with formalized toxoid preceded, followed or mixed with Restim. A significant increase in protecting antibodies was observed where the two substances were injected simultaneously. Injection of Restim before or after toxoid administration produced equivocal results. To rule out the influence of the species of animal used, the procedures were repeated with pheasants with similar results. The data obtained will be discussed from the standpoint of the effect of Restim on the antibody producing mechanism, the chemical nature of Restim, and the appearance of circulating antibodies in relation to time.

8 *Immunology*

Bovine serum inoculation of chickens at different ages and its effect on the secondary response. Harold R. Wolfe, A. Amin* and August P. Mueller*. University of Wisconsin, Madison, Wisconsin.

Chickens were given a single primary inoculation of 40 mgs of bovine serum albumin per KBW at either 20 days or 6, or 12 weeks of age. A single secondary injection of 40 mg per KBW was given at 6, 12 and 22 weeks. Bleedings were made on several days beginning with the 4th day following the injection. The antibody nitrogen of the circulating blood was determined as was the disappearance of the antigen. The primary inoculation at 20 days did not sensitize the birds and no secondary response was evident at 6, 12 or 22 weeks of age. Only a few of the chickens given a primary inoculation at 6 weeks gave a secondary type of response when challenged at 12 or 22 weeks of age. A good secondary type of response was elicited in 22 week old chickens if the primary injection was given at 12 weeks of age. The results indicate a certain maturity of the immune mechanism must be present in order for the chicken to be able to be sensitized to give a secondary type of response. (Supported by a grant from the National Science Foundation)

9 *Immunology*

EFFECT OF APPENDIX-SHIELDING DURING BODY X IRRADIATION ON SPLENIC WHITE-PULP RECOVERY IN HEMOLYSIN-FORMING RABBITS. Dieter H. Sussdorf (intr. by J. S. Garvey). Argonne Ntl. Laboratory, Lemont, Ill. and Calif. Inst. of Tech., Pasadena.

A greater rate of splenic white-pulp recovery and a near-normal hemolysin response were observed when the appendix was shielded during body X irradiation of rabbits (J. Infect. Dis. 105:238, 1959). The hypothesis that the spleen was repopulated by lymphoid cells of the shielded appendix was tested. Rabbits were injected with tritiated thymidine and sheep red cells 17 and 24 hours, respectively, after receiving a body dose of 500r with or without appendix-shielding. Radioautographic analysis of organ touch preparations revealed that in the appendix-shielded group, 20% of the lymphoid cells of the appendix were labeled 3 hours after thymidine injection, while labeled cells did not appear in the spleen and a mesenteric lymph node until 1 day later. Division caused a rise of the percentage of labeled cells to 27% in the appendix and 18% in the spleen. In the totally irradiated controls, the percentages rose from an initial 5% to 7% in the appendix, and from 1% to 3% in the spleen. Since the DNA-specific thymidine is available for incorporation for only about 2 hours after injection, the delayed appearance and subsequent marked increase in number of labeled cells in the spleen after appendix-shielding lend support to the repopulation hypothesis. (Work performed in part under the auspices of the U. S. Atomic Energy Commission.)

10

Immunology

A ROLE FOR PHOSPHOLIPIDES IN THE UPTAKE OF AMINO ACIDS BY LYMPH NODE CELLS. Herman Friedman* and William L. Gaby. Albert Einstein Med. Center and Hahnemann Med. Coll., Phila., Pa.

A metabolic role for phospholipids in the incorporation of amino acids by Penicillium mold cells has been demonstrated while a similar role for lipids in the uptake of amino acids by mammalian and avian tissue in vivo and in vitro has been recently described. The possible role of lymph node cell phospholipid fractions in the incorporation of amino acids in vitro by immune and normal lymph node cells has been studied. Incubation of C^{14} labeled amino acids with washed cell suspensions of rabbit lymph node cells obtained from the popliteal and axillary nodes either from animals hyperimmune to Shigella paradysenteriae antigens or from normal control animals resulted in a rapid uptake of the labeled amino acid from the incubation media. No significant difference could be noted between immune and normal control cells, even in the presence of antigen. Fractionation of aliquots of cells at varying intervals during incubation indicated a rapid accumulation of radioactivity in the phospholipid portion of the cells during the initial period of incubation and a gradual uptake by other fractions. Similar results were obtained with larger volumes of bovine lymph node cell suspensions incubated with lysine-C^{14}, glycine-C^{14}, leucine-C^{14}, or valine-C^{14}. The labeled amino acids could not be separated from the purified phospholipid fraction.

11

Immunology

IDENTIFICATION OF IODINE-REACTING AMINO ACIDS IN ANTIBODY. Marian Elliott Koshland, M. J. Erwin* and F. M. Englberger.* Biology Dept., Brookhaven National Laboratory, Upton, N. Y.

It has been shown that the uptake of iodine by pure preparations of antihaptene antibody and gamma globulin is identical and that saturation is achieved by the binding of 105 I atoms/mol of protein. To determine whether the same residues were involved in the iodine uptake, the amino acid content of both iodinated and uniodinated antibody and globulin was assayed. The following results were obtained: (1) The amino acid content of 4 different preparations of anti-haptene antibody was similar to that of anti-carbohydrate antibody; (2) No significant difference was found between the amino acid content or the iodine-reacting residues of antibody and gamma globulin; (3) In the surface-saturated proteins 46 of the 59 tyrosine residues and 6 of the 16 histidine residues were iodinated, which on the basis of 2 I atoms/amino acid accounts for the observed I atoms bound at saturation; (4) In addition, 6 of the 21 tryptophane residues and 11 of the 15 methionine residues were destroyed by iodination. The reacted methionine appeared quantitatively as methionine sulfoxide. Studies of partially iodinated proteins have shown that the tryptophane and methionine react more slowly than the tyrosine and histidine. The significance of these results in relation to the amino acids involved in antibody action will be discussed. (Supported by USAEC.)

12

Immunology

HISTOLOGIC STUDY OF THE SECONDARY RESPONSE IN THE RABBIT P. A. Ward*, A. G. Johnson, and M. R. Abell*, Dept. of Bacteriology and Dept. of Pathology, Univ. of Michigan, Ann Arbor.

Histologic changes in various rabbit tissues were studied following 2 intravenous injections, spaced 6 weeks apart, of 10 mg bovine gamma globulin. Groups of 3 rabbits were sacrificed 1, 2, 4, 6, 8, 10, 12, and 15 days after the 2nd injection. Hemagglutinin titers rose abruptly to a peak on the 4th day, maintained this high level through the 10th day, but decreased considerably on the 12th day. As in the primary response (J. Exp. Med. 1959, 109, 463), the numbers of mitotic figures and pyroninophilic reticular cells within splenic follicles were marked but appeared earlier. They were maximum on days 4 and 6, but much less on day 8, and near normal on day 10. In contrast to the primary response, 2 injections of BGG showed (a) significant pyroninophilic reticular cells and large lymphocytes in the red pulp of the spleen, (b) numerous plasma cells in the splenic medullary cords during the final stages of the response, and (c) considerable activity in the germinal centers of the lymph nodes. The latter were composed of colonies of pyroninophilic reticular cells and were largest on the 6th day. They had decreased considerably on the 8th day. The medulla of the lymph nodes increased from the 2nd through the 12th day, due primarily to modified reticular cells and large and medium sized lymphocytes.

13

Immunology

DIFFERENCES IN THE PRIMARY STRUCTURE OF RELATED PURIFIED ANTIBODIES. David Gitlin. Dept. of Pediatrics at the Children's Hospital, Harvard Medical School, Boston, Mass.

Rabbit antibodies against pneumococcus polysaccharides of type 6, 7 and 12 were isolated by interaction with their homologus antigens and then denatured at 60°C or 100°C. Enzymatic digestion of the proteins was performed in the pH-stat using either subtilisin, trypsin, chymotrypsin or pepsin or combinations of these enzymes. The resulting peptides were separated two-dimensionally on filter paper using high voltage electrophoresis for one dimension and n-butanol/acetic acid/water for the other. It was found that many of the peptides present in one antibody were not present in the others and vice versa. Thus, at least these three antibodies differed markedly in their primary structure or amino acid sequence, and hence could not have been formed by refolding of a polypeptide chain with a single primary structure as suggested by Pauling (J. Am. Chem. Soc. 62:2643, 1940). In addition, if the primary structure of a protein is genetically determined and the primary structure of an antibody differs for each specific antigen, it is clear that genetic factors may be involved in the induction of antibody synthesis by antigens. It is also clear that the heterogeneity of the γ-globulins extends to the primary structure of each molecular species.

14

Immunology

THE EXHAUSTION OF SPECIFIC ANTIBODY-PRODUCING CAPACITY DURING A SECONDARY ANTIBODY RESPONSE. E. Sercarz* and A. H. Coons, Dept. of Bacteriology and Immunology, Harvard Medical School, Boston, Mass.

Mice of the Harvard strain (not inbred) produced an antibody response to 2 mg of alum-precipitated BSA, first detectable after three weeks by tanned red cell agglutination. Subsequent transfer of their spleens and lymph nodes with simultaneous injection of antigen into normal mice led to a high level of antibody one week later in 15 of 20 recipients. However, if the donors had been injected with a second dose of antigen 6 to 11 days before transfer, there was antibody formation in only 4 of 19 recipients seven days after transfer and antigen injection. This failure to respond suggests that the second injection of antigen expends the sensitive cells by stimulating their differentiation along an irreversible pathway leading to antibody formation. We presume that subsequent antigenic challenge can set off antibody synthesis only in other cells which receive their primary stimulus as a result of the second injection of antigen. Evidently, the accumulation of sufficient, new sensitive cells to transfer an appreciable antibody response requires more than 11 days. In support of this explanation, preliminary results indicate that transfer again becomes successful 14 days after the donor's secondary challenge.

15

Immunology

QUANTITATIVE ASPECTS OF ORAL IMMUNIZATION AGAINST BOVINE SERUM ALBUMIN (BSA). Richard S. Farr, Winifred Dickinson,* and Keith Smith.* Univ. of Pittsburgh School of Medicine.

Adult rabbits were fed 0.000025-0.25% solutions of BSA as drinking water and the daily intake averaged 250 ml. Antibody responses were measured with the ammonium sulfate technique (J. Inf. Dis., 103:239, 1958). The minimal oral immunizing dose of BSA ranged from 0.0025% for 30 days to 0.25% for 1 day. Antibody was detected as early as 7 days after the first feeding of 0.1% BSA. The concentration of BSA or haptenic BSA fragments in the circulation during a 5 day course of immunization with 0.1% BSA was measured by the capacity of sera to inhibit the binding between known amounts of anti-BSA and I^{131}BSA. This method can detect as little as 0.005γ unlabeled BSA N/ml serum. It was found that at no time during oral immunization with 0.1% BSA did sera contain as much BSA as was present in sera of rabbits 7 days after an i.v. injection of a non-antigenic dose of 6γ BSA N. Since the i.v. injected animals were not immunized despite the fact that the spleen, bone marrow, and peripheral lymphoid tissues were exposed to higher concentrations of BSA than were similar tissues of the orally immunized animals, it is suggested that immunization by the oral route was initiated by lymphoid tissue associated with the intestinal tract where higher concentrations of BSA were available.

XUM

16 *Immunology*

INTERFERENCE WITH IMMUNE RESPONSES TO HEMO-ANTIGENS. Kurt Stern. Depts. of Path., Chicago Med. Sch. and Mt. Sinai Hosp., and Mt. Sinai Med. Res. Foundn., Chicago, Ill.

Isosensitization to the Rh factor is significantly suppressed when sensitizing red cells are ABO-incompatible with the recipient. Since the mechanism of this inhibition was never conclusively explained, model experiments were devised in order to study possible interference with antibody formation of two antigens located on the same red cell. In the first experimental design natural antibody for human A red cells in rabbits was stimulated by injection of papainized A cells. One group of rabbits was then injected with ON and the other with AN cells. The immune serums were titrated before and after suitable absorptions with ON, AN, OM, AM. Significantly higher anti-N titers were found in rabbits injected with ON cells than in those injected with "incompatible" AN cells. In the second experimental design rabbits immunized with human globulin and injected with sheep red cells coated in vitro with human globulin produced lower sheep hemolysin titers than similar rabbits injected with non-coated cells. Thus immune responses appear to be inhibited when the host possesses antibodies capable of reacting with another antigen present in or on the red cell envelope. (Supported in part by a grant from the Natl. Inst. Health, RG-5339.)

17 *Immunology*

ROUTES OF ANTIGEN ADMINISTRATION IN PRODUCTION OF EXPERIMENTAL ALLERGIC ENCEPHALOMYELITIS AND ANTIBODY. R.M.Condie*, D. Youel* and R.A. Good. University of Minnesota, Minneapolis.

The intradermal route favors development of delayed hypersensitivity while intravenous injection stimulates production of circulating antibody. Intradermal route of injection favors development of EAE. It has been suggested that since EAE and delayed sensitivity are both favored by the I.D. route, delayed allergy is responsible for EAE. Studies were undertaken to determine the effect of various routes of BSA adjuvant injection on amount of precipitating antibody produced. The routes included I.D. foot pad, I.D. back, S.Q. back, I.P. and I.V. The amounts of antibody produced were established by quantitative precipitin tests carried out on sera obtained at 5,7,8, 14,22 and 38 days following injection of antigen. Following injection by the I.D. foot pad route, the amount of antibody produced far exceeded that produced by any other route. Surprisingly when intradermal injection was given into the skin of the back scarcely any more antibody was produced than when injections were given subcutaneously and intraperitoneally. The intravenous route was intermediate in effectiveness. These results parallel in a striking way the results obtained when incidence of paralysis produced by injection of spinal cord adjuvant by these same routes were compared. (Aided by grants from U.S.P.H.S., Minn. Heart, American Heart Association)

18 *Immunology*

PREVENTION OF SECONDARY IMMUNE RESPONSE WITH 6-MERCAPTOPURINE E. S. LaPlante and R. M. Condie (intr. by R. A. Good) Pediatric Research Labs., Univ. of Minnesota, Minneapolis, Minnesota.

Primary immune response has been inhibited with 6-MP in a dosage of 6 mg./kg. in rabbits. This same dose only delays appearance of circulating antibody during the secondary response. The present study with 6-MP is concerned primarily with its effect on the secondary immune response. Rabbits received primary stimulation with 20 mg. bovine serum albumin (BSA) I.V. and were bled 14 days later to determine which animals had responded to this antigenic stimulation. Those animals in which a primary response had been demonstrated and which showed no remaining antibody 30 days later were then stimulated with 20 mg. BSA I.V. and divided into 3 groups. Group I was treated with 15 mg./kg. 6-MP daily for 10 days, Group II with 12 mg./kg. daily for 8 days. Group III served as controls. The dosage of 12 and 15 mg./kg./day prevented antibody formation in the secondary response in most of the animals. Prolonged pretreatment with 6-MP in a dosage of 6mg./kg. failed to prevent the secondary response. Preliminary data suggest that animals in which 6-MP prevented the secondary response also failed to respond to tertiary antigenic stimulation with BSA even though they were given no further treatment with 6-MP. (Supported by grants from U.S.P.H.S., Minnesota Heart Association, and American Heart Association).

19 *Immunology*

FACTORS GOVERNING ANTIBODY PRODUCTION IN NEWLY-HATCHED CHICKS BY TRANSFERRED IN VITRO STIMULATED ADULT HOMOLOGOUS SPLEEN CELLS. B.W. Papermaster and S.G. Bradley (intr. by Dennis W. Watson) Univ. of Minnesota Medical School, Minneapolis, Minn.

Previous studies have demonstrated antibody production to B. abortus antigen in neonatal chicks 6 days after transfer of homologous, normal adult chicken spleen cells mixed with antigen in vitro (Trnka and Riha, Nature, 1959; Papermaster et al, Proc. Soc., 1959). In order to study further the variable reactivity of recipient chicks they were splenectomized 24 hours before receiving adult cells mixed with antigen. A second group was injected with Thorotrast I.V. 24 hours prior to cell transfer. In both treated groups titers were higher than in untreated recipients, suggesting that these operations tended to inhibit host resistance to proliferating donor cells. Moreover, it has also been possible to pass the antibody producing capacity serially in the neonatal host following single and repeated antigenic challenge. Histological evidence also supports the following conclusions: 1) the neonatal chick will support growth and function of in vitro stimulated adult cells, but will not respond to direct antigenic challenge with B. abortus 2) procedures altering the total available reticuloendothelial system favor the establishment and function of the transplant. (Supported by grants from the U.S.P.H.S., Minnesota and American Heart Associations)

20 *Immunology*

SYNTHESIS AND RELEASE OF ANTIBODY BY ISOLATED LYMPH NODE CELLS. E. Heimreich*, M. Kern and H. N. Eisen, Washington University School of Medicine, St. Louis.

Cells from lymph nodes of rabbits, injected with 2,4-dinitrophenyl bovine γ-globulin (DNP-BγG), were incubated with C^{14} amino acids. Cells and medium were then separated. Cell lysates and extracellular fluids were cleared in an ultracentrifuge and assayed for C^{14}-labeled antibody. A rapid assay was provided by measurement of C^{14} specifically extracted by haptens from precipitates made with carrier anti-DNP antibody and DNP-BγG. Indistinguishable results were obtained by C^{14} counting of homologous specific precipitates, prepared after removal of non-specific C^{14} by repeated precipitations with an unrelated immune system. Cells from control animals (not injected with DNP-BγG) yielded $< 5\%$ of the C^{14}-antibody of cells from DNP-BγG injected animals. C^{14}-labeled antibody appears in cells without a lag, reaches a maximum after 60' and remains constant thereafter. Antibody in the outside fluid appears only after about 20' and then increases linearly for 4-5 hrs. Anaerobically and at reduced temperatures, synthesis and release were both markedly reduced. Experimental distinctions between (a) a specific translocation mechanism, (b) cytoplasmic shedding, and (c) cell lysis, as possible mechanisms by which cells release antibodies, will be presented.

138. IMMUNE RESPONSE–Antigen–Antibody Reaction

1 *Physiology*

16 MM Motion Picture
(Sound)

CELLULAR CHANGES LEADING TO THE FORMATION OF PLASMA CELLS.

By B. Bryant and Y. Osada
(introduced by J. B. Hamilton)

State University of New York

Department of Anatomy

450 Clarkson Avenue

Brooklyn 3, N. Y.

This movie illustrates successive changes in the cellular responses to an injection of antigen in an immunized animal. The progressive changes in neutrophils, eosinophils, macrophages, transitional and plasma cells are shown both in stained preparation and in phase microscopy.

2 *Biochemistry*

THE FUNCTION OF THE N-TERMINAL LYSINE IN RIBONUCLEASE IMMUNE REACTIVITY. Ray K. Brown. Div. of Laboratories and Research, New York State Dept. of Health, Albany.

Chemical modification of the α amino group of guanidinated RNase or removal of the N-terminal amino acid from guanidinated RNase by the Edman procedure yields derivatives which react only partially with rabbit antiRNase. Recently Baker and King have purified by countercurrent distribution a RNase lacking an N-terminal lysine. This des-lysino RNase reacts only partially with antibody. Thus, the α amino group and perhaps more of the N-terminal lysine are necessary. The α amino group might be an actual site of union of antigen and antibody or it might stabilize the configuration of the portion of the RNase which reacts with antibody. If it is a site of sufficiently strong union, a complex of RNase with its antibody should label poorly with fluorodinitrobenzene. After careful controls were done to correct for loss, as much α-ε-bis-DNP lysine was recovered from RNase antiRNase as from a mixture of RNase and non-specific rabbit γ globulin. Binding studies also do not give evidence of interaction of antiRNase and lysine. These data suggest that the N-terminal lysine stabilizes an antigen configuration rather than reacts with antibody. (Supported in part by Grant E1846 from the National Institutes of Health.)

3 *Biochemistry*

IMMUNOLIGICAL INVESTIGATION OF RAT UTERINE FLUID. Henry J. Albers and M. Neves e Castro (Gulbenkian Fellow) (intr. by David Stone.) Worc. Found. Exp. Biol. Shrewsbury, Mass.

Rat uterine fluid collected during the proestrus stage was studied by immunoelectrophoretic analysis and the Ouchterlony technique. Antibodies against rat uterine fluid and serum were produced in rabbits using Freund adjuvant. The immunological and electrophoretic behavior of rat uterine fluid, rat serum and rabbit anti-sera was not affected by lyophilization, and ligation of neutero-tubal junction and cervix prior to collection of the uterine fluid did not alter the characteristic behavior. This fluid gave rise to two precipitation lines with β-globulin mobility and one line with albumin mobility against anti-uterine fluid serum. When this serum was absorbed with rat serum it still reacted with the uterine fluid giving rise to one of the lines with β-globulin mobility. Results suggest that the uterine fluid is not a serum filtrate and that the uterine wall secretes a component that is antigenically different from the normal serum components. (Supported by a grant from the Planned Parenthood Federation.)

4 *Pathology*

CUTANEOUS REACTIONS IN GUINEA PIGS INJECTED WITH RABBIT ANTI-GUINEA PIG ERYTHROCYTE SERUM. Changling Lee* and Israel Davidsohn. Depts. of Pathology, Chicago Medical School and Mount Sinai Hospital, Chicago, Illinois.

We reported previously that normal rabbits injected intracutaneously with anti-rabbit erythrocyte serum showed local reactions characteristic grossly and microscopically (Arch. Path. 54: 540, 1957). The same was observed in guinea pigs injected intracutaneously with rabbit anti-guinea pig erythrocyte serum. The amount and titer of the injected serum and the interval following injection were factors determining the intensity of the reaction. Three types of lesions different in appearance were observed in: (1) normal animals; (2) in animals previously injected with normal rabbit serum; and (3) following injection of serum containing Forssman antibodies. The factor in the serum responsible for the lesions could be removed by erythrocytes, to a lesser extent by leukocytes and not at all by homogenates of guinea pig skin. It was thermostable up to 70C but was destroyed at 75C. It seemed to consist of small molecules. With electrophoretic fractions of the serum, skin lesions were produced only with fast moving gamma-2 globulin fractions while all gamma-2 fractions caused anemia. (Supported in part by grant A-77 of the National Institutes of Health, United States Public Health Service and by the Mount Sinai Medical Research Foundation).

5 *Pathology*

ANTIGENICITY OF TROPOCOLLAGEN. Roy E. Ritts and John C. Houck. Depts. of Microbiol. and Biochem., Georgetown Univ. Schools of Medicine and Dentistry, Washington 7, D.C.

Recent studies have indicated that only complement fixing antibodies are produced in rabbits by repeated injections of rat collagen-complete adjuvant mixtures. In this study a mixture of chemically pure calf skin tropocollagen (10 mg/dose) and complete adjuvant (1 mg Myco. butyricum /initial dose) was injected i.m. into thirty 4-5 lb. albino rabbits for 4 doses at 7-10 day intervals with progressive halving of the amount of tubercle bacilli per dose. Bleedings 2wks. to 4mos. later revealed precipitins in 27 of the rabbits when tested against the original tropocollagen but not against bovine gamma globulin or albumin. Similar studies in agar revealed a single band of precipitation against tropocollagen except in 5 of the reacting animals where the band was split in the center area. None of the reactors showed agglutination for rheumatoid factor as tested by the human Fraction II-bentonite technique. Control animals consisted of 48 rabbits injected with complete adjuvant containing comparable amounts of tubercle bacilli. None of these possessed tropocollagen antibody. All animals gave delayed-type skin reactivity to 0.005 mg of PPD. Eleven of the antibody-possessing rabbits exhibited an Arthus-like skin reactivity to 0.1 and 0.5 mg of tropocollagen. There was no evidence of renal, hepatic or splenic damage.

6 *Pathology*

AN EFFECT OF ANTIGEN-ANTIBODY COMPLEXES AND ENDOTOXINS ON LEUCOCYTE METABOLISM. B. Strauss (intr. by C.A. Stetson, Jr.). N.Y.U. College of Med., New York.

Heparinized whole blood in Warburg vessels exhibits a steady uptake of oxygen due to the respiratory activity of the leucocytes. Addition of bacterial endotoxins (0.1-100 µgms/ml) produces an abrupt and marked increase in the rate of oxygen consumption. This effect has been demonstrated with blood of man, monkey, dog, and guinea pig, but not with that of rabbit, chicken or bull. The capacity of leucocytes to react to endotoxin is retained for many hours in Warburg vessels, but is greatly reduced or abolished by such manipulations as difibrination or centrifugation. The effect of endotoxin is blocked by cyanide, fluoride, iodoacetate or citrate. The oxygen consumption of canine thoracic duct lymph and of isolated human lymphocytes is not affected by endotoxin. Determinations of leucocyte glycogen before and after addition of endotoxin indicate that the substrate for the increased oxygen consumption is not derived from this source. Similar effects are observed upon addition of antigen and antibody in sequence or on addition of antigen-antibody complexes. This model system provides a sensitive new method for the study of the nature of the biological activity of these substances.

7 *Pathology*

MECHANISM OF IMMUNE LYSIS OF TUMOR CELLS. Anton Lindner. Depts. of Pathology, VA Hospital and Univ. of Buffalo School of Medicine, Buffalo, N. Y.

Anti-sera produce a cytotoxic effect on ascites tumor cells. Mice bearing Ehrlich Ascites tumor were given 2 mg immune gamma globulin (obtained by ethanol fractionation of sera from rabbits immunized with whole Ehrlich Ascites tumor cells) in 1 ml guinea pig complement on the 4th, 5th and 6th days after tumor innoculation. Control mice were injected with non-immune gamma globulin and complement or saline. The tumor cells were stained with histochemical methods for lipids, carbohydrates, nucleic acids and proteins. Different degrees of cytotoxic changes were seen in the same tumor population. The cells with the least amount of damage showed cytoplasmic swelling and formation of bubbles at the cell surface, apparently due to antigen-antibody reaction at the surface (Lindner, Fed. Proc., 17:446, 1958). These cells also showed loss of cytoplasmic RNA and protein. 25% of the cells showed more severe damage such as almost complete lysis with some loss of nuclear DNA. In general, there was good correlation between structural and metabolic changes. (Supported by a grant from the National Cancer Institute.)

XUM

8

Pathology

THE EPINEPHRINE LESION IN HYPERSENSITIVITY. Robert R. Gatling (intr. by Joel G. Brunson.) Path. Dept., Univ. of Miss. Med. Center, Jackson, Miss.

Intradermal injection of epinephrine (or norepinephrine) in hypersensitive rabbits in the presence of circulating specific antigen results in the production of a characteristic and reproducible local lesion. The intensity of the lesion is directly related to the degree of sensitivity of the rabbit as measured by the Arthus reaction and, qualitatively, is similar to the latter in that it embodies the features of edema, congestion, hemorrhage, cellular exudation and fibrinoid necrosis of arteries. Heparin or cortisone completely inhibits or greatly ameliorates the reaction; nitrogen mustard, chlorpromazine, or sodium salicylate exert no measurable inhibition. Ephedrine, histamine and atropine injected into the skin under similar conditions produce no constant lesion. This method is offered as a means of producing lesions at predetermined foci in hypersensitive animals which might be useful as an investigative tool for promotion of a better understanding of mechanisms involved in the production of focal lesions which seem to be related to the hypersensitive or allergic state. The conditions surrounding the production of the epinephrine lesion have much in common with those postulated to exist when focal lesions occur during hypersensitivity in the human.

9

Immunology

SEROLOGICAL STUDIES OF OVALBUMIN DENATURED BY γ-RAYS. Charles A. Leone. University of Kansas, Lawrence, Ks.

Radiation-denatured, isoelectrically insoluble fractions, derived from samples of ovalbumin that had absorbed various doses of γ-rays, were serologically equivalent when quantitatively compared by means of precipitating antisera against the radiation-denatured ovalbumin. Ovalbumin that had been denatured by heat, guanidine hydrochloride, urea, ethanol, and acetone all gave strong cross reactions when tested with these same antisera. The qualitative nature of the cross reactions of the heat-, guanidine-, and urea-denatured samples was analogous to those that are obtained among naturally cross-reacting proteins. Ethanol- and acetone-denatured samples appeared to be more extensively damaged and reacted only at much higher ranges of antigen concentrations. Reciprocal tests with antisera produced against heat-denatured and urea-denatured ovalbumins confirmed the high serological correspondence among the variously denatured ovalbumins including the radiation-denatured protein. (Supported by USPHS grant E-1809 and AEC grant AT(11-1)-83.)

10

Immunology

COAGULATED SPECIFIC COMPLEXES AS ADSORBENTS FOR ANTIGEN. G. Bonar Sutherland (intr. by Charles Leone.) Dept. of Physiology, Univ. of Kansas, Lawrence, Kansas.

It has been found that if rabbit antibody or antigen-antibody complexes are coagulated under carefully controlled conditions by any of the following reagents: aluminum chloride, chromium chloride, acetone, ethanol, hydrochloric acid, sulfuric acid, or heat, such antibodies or complexes will serve as specific adsorbents for antigen. The antigen may be subsequently released at pH 3 and the coagulum reused at least 5 times. The best yield thus far obtained indicated that only about 5% of the antibody combining sites were available, however, this represents a practical quantity: about three-tenths milligrams of ovalbumin was obtained per elution when 10 ml. of specific antiserum were used in forming the coagulum. The technique may prove useful in the isolation of tissue-specific proteins, viruses or toxins.

11

Immunology

ARTIFACTS IN GEL DIFFUSION DUE TO CONCENTRATION OF AND DISTANCE BETWEEN ANTIGEN AND ANTIBODY. Sung J. Liao. Waterbury Hospital, Waterbury, Connecticut.

Gel diffusion technique has been used to analyse bact. and tissue antigens. The various components of an antigen form distinct lines with its antibody in agar gel. It is generally accepted that an antigen shall form a definite number of lines with its antibody and it can, therefore, be specifically identified. In our hands, this supposition could not be substantiated. It seems that concentrations of reagents and distances between them on agar plate have marked influence on the number of lines. Variations of these two factors may make the same reagents appear to be totally different materials. For our investigation, 1% agar in saline was autoclaved and congealed in sterile petri dishes. Sterile pyrex glass cylinders were placed on agar as containers of reagents. Two antigen-antibody systems were used, viz., diphtheria toxoid-antitoxin and pneumococcus polysaccharide-its antiserum: in two conc., i.e., original and 1:10. Preparations were observed at 37C for 4 days and then at 9 C for 6 more days. When they were 1 cm apart, diluted antiserum formed more lines with conc. antigen than conc. antiserum with conc. antigen. The same pair of AG-AB system formed more lines when 1 cm apart than when 2 cm apart. At the same distance, the conc. pair formed more lines than diluted pair of the same system.

12

Immunology

SEROLOGICAL CORRESPONDENCE AND ZONE DISPLACEMENT IN COMPLEX PRECIPITATING SYSTEMS. Robert K. Jennings and Esther Arditi*. Chicago Medical School, Chicago.

Studies about to be published attribute displacement of the heterologous zone in complex precipitating systems to the influence of antigen formed from the homologous member of the mixture by occlusion of determinant groups specific for the interzonal antibody. It was suggested that this antigen might be a congener of the homologous antigen or a product formed with the interzonal antibody. It was also suggested that displacement may be more pronounced when the heterologous antigen is not closely related to the homologous antigen serologically.

The present experiments demonstrate that the degree of serological correspondence of the antigens in a complex precipitating system does affect the amount of zone displacement. The relationships are consistent with the conclusion that the displacement is due to complex acting as a secondary antigen rather than to heterologous antigen coexisting with the homologous antigen before diffusion.

Supported by a grant, E-2866, from the USPHS.

13

Immunology

TISSUE ANTIBODY STUDIES. Louis H. Muschel, Lois A. Simonton* and Patricia A. Wells*. Dept. of Serology, Div. of Communicable Disease, Walter Reed Army Institute of Research, Wash., D. C.

A complement fixation test was developed for detecting antibodies against saline extracts of calf thymus and rabbit liver. The extract antigens were used in amounts containing about 5 mg protein or less. They were not anticomplementary against 5 C'H50 during fixation at 4°C for 16 hours. Reactivity of the antigens was destroyed by boiling 5 minutes, but preserved at -20 C for at least 30 days. Normal sera of rabbits and monkeys had low levels of tissue antibodies in contrast to normal sera of humans, rats and mice which were nonreactive. Rabbits infected with trypanosomes developed significant rises in tissue antibody. On the other hand, rabbits inoculated with Treponema pallidum which developed high titers of T. pallidum immobilizing and Wassermann antibodies did not respond. Other attempts to induce increased levels were not successful: inoculation of rabbits with several bacterial and rickettsial vaccines, burns in rats, X-irradiation of mice, and a stress situation in a monkey leading to a duodenal ulcer. Sera of normal Wassermann negative humans have been nonreactive. Sera of patients with certain infectious diseases and those in which immunological mechanisms are implicated, particularly systemic lupus erythematosus, have reacted. The role of these antibodies remains to be determined.

14 *Immunology*

ADAPTATION OF CHICK EMBRYO CULTURES TO IMMUNOLOGICAL STUDIES. R. F. Ruth (intr. by T. Makinodan.) Dept. of Embryology, Carnegie Inst. Wash., Baltimore, Md.

Mun's report (Biol. Bull. 115: 239, 1958) that the toxic effects of heated rabbit antisera upon the chick embryo *in ovo* may be enhanced by sources of mammalian complement and properdin, led to *in vitro* studies (Carnegie Inst. Wash., Year Book 58, 1959). Undiluted mammalian sera frequently kill the early embryo. Heating at 56° C for 20 minutes reduces this effect. The use of mixtures of differently derived and treated sera was complicated by the occasional better development in diluted sera as compared to saline controls. Because the sera, which separate the embryo from the atmosphere, must contain hemoglobin and carbonic anhydrase due to hemolysis, these two proteins were added separately to saline cultures. 0.4% Carbonic anhydrase restored the development of the nervous system, the somites, and the heart beat. Consistent and synchronous development, comparable to the first 5 days *in ovo*, has been obtained under liquid by use of a 95% O_2 - 5% CO_2 atmosphere. This has made practical the statistical study of the effects of mixtures of fluid sera, in direct contact with the embryo.

15 *Immunology*

CONVERSION OF PRECIPITATING TO NON-PRECIPITATING ANTIBODY. **Jacob J. Prusansky*, Samuel M. Feinberg, and Norma Wojcik.*** **Northwestern Univ. Med. School, Chicago, Ill.**

Heat inactivation at 56°C of the complement of potent guinea pig antisera results in a virtually complete loss of precipitating ability of the sera. In passive anaphylaxis, however, the minimum sensitizing dose of heated serum is only slightly higher than that of unheated serum. Prior heating of antisera at 37°C or at 46°C for 30 minutes does not measurably reduce the amount of precipitate formed in the presence of antigen. Complement-fixed serum contains normal amounts of precipitin. The ability of heated serum to precipitate in the presence of specific antigen is not restored by the addition of even large amounts of fresh normal guinea pig serum. Precipitin curves of mixtures of heated and unheated serum indicate that a considerable amount of coprecipitation occurs. Heating at 56°C converts guinea pig precipitating antibody into coprecipitating antibody which retains the ability to passively sensitize guinea pigs to systemic anaphylaxis. (Supported by U.S.P.H.S. Grant No. B-1226).

16 *Immunology*

THE DEGRADATION OF I^{131} LABELED IMMUNE γ GLOBULIN IN MICE. **Geronimo Terres (intr. by Ruth Drew.) Brookhaven Nat'l Lab., Upton, N.Y.**

The degradation of administered I^{131} labeled immune rabbit γ globulin (anti-bovine serum albumin antibody: anti-BSA) was followed in mice by whole body measurement of radioactivity. The per cent of γ globulin degraded at an accelerated rate in the first 24 hours was studied as a function of the amount of BSA concomitantly injected with the γ globulin. The same I^{131} γ globulin - BSA ratios also were tested *in vitro* and the per cent of radioactivity precipitated determined. In antibody excess the per cent of γ globulin precipitated *in vitro* was the same as the per cent degraded at an accelerated rate. From equivalence (*in vitro*) into slight antigen excess the per cent of I^{131} labeled γ globulin precipitated decreased while the per cent degraded at accelerated remained constant. In antigen excess (30 x amt. of BSA necessary for maximum precipitation) the per cent of γ globulin degraded at an accelerated rate also decreased with an increase in the amount of BSA injected. At approximately 1000 x the amount of BSA necessary for maximum precipitation, none of the γ globulin was degraded at an accelerated rate. The significances of these results will be discussed.

17 *Immunology*

EFFECT OF DIFFERENT "CONTACT CONDITIONS" ON THE BLUETONGUE VIRUS-ANTIBODY REACTION. **S.-E. Svehag (intr. by J. R. Gorham.) College of Vet. Med., Washington State University, Pullman, Wn.**

Regressions of neutralized BTV (bluetongue virus) on serum titers were plotted for varying "contact conditions" (time and temperature factors for incubation of the virus-serum mixture), and the kinetics of BTV neutralization at 37°C. by immune ovine and bovine sera was studied. The regression lines were fitted to experimental data calculated from neutralization tests in suckling mice, chicken embryos and cell culture. (1) Relative serum titer values increased ±100 fold when a more favorable "contact condition" (2½ hours at 37°C.) was compared with conventional methods (i.e. 1 1/2 hours at room temperature). The regressions were linear and significant ($P < 0.001$) for virus concentrations above 25 LD_{50} or ID_{50}. Below this value the serum became relatively less efficient when diluted and the "percentage law" did not fit the data. This effect gradually disappeared in the regressions in which the time and temperature of incubation were approaching optimal "contact conditions." (2) The kinetics of BTV neutralization at 37°C. revealed an early and a late serum action, which might suggest the presence of antibodies of high and low avidity. (3) A heat stable inhibitor directed against BTV was found in normal sera of certain mammals. The degree of inhibitory effect was altered by applying different "contact conditions."

18 *Immunology*

SEROLOGIC BEHAVIOR OF RICKETTSIAL ANTIGENS AFTER PURIFICATION BY ANION EXCHANGE CHROMATOGRAPHY. Norman K. Brown (Introduced by Henry S. Fuller). Walter Reed Army Institute of Research, Washington, D. C.

Cellulose anion exchange chromatography has been used to purify from yolk sac suspensions specific complement fixing antigens of the following agents: *Rickettsia mooseri*, *R. prowazekii*, *R. sibiricus*, and the unnamed agent of Indian tick-typhus. The *R. sibiricus* antigen did not react with antibody to Fievre boutonneuse or Indian tick-typhus; by contrast, the Indian tick-typhus antigen reacted with antibody against Fievre boutonneuse but not that against *R. sibiricus*. These findings make it reasonable to classify the Indian tick-typhus agent as a strain of *R. conorii*, which is distinct from *R. sibiricus*.

Purified typhus group antigens have been studied for their sedimentation properties in sucrose density gradients. Chromatographic columns bearing these two respective antigens were employed to absorb homologous and heterologous antisera. Factors affecting the properties of residual antibody in the effluent included physical structure of the column, rate of flow, and relative concentrations of reactants.

19 *Immunology*

THE ANTIGENICITY OF ESTRONE-PROTEIN CONJUGATES. L. Goodfriend* and A.H. Sehon, Departments of Biochemistry and Chemistry, McGill University, Montreal, Quebec.

The aim of the present study was to determine whether steroids, when conjugated by stable, covalent bonds to proteins could act as haptenic groups in inducing steroid-specific antibodies. For this purpose, estrone was converted to estrone-17-isocyanate and the latter was reacted with serum proteins to form estrone-17-carbamido-protein conjugates. Electropnoretic, ultraviolet and chemical analyses showed that 20 to 25 estrone residues were coupled per molecule of human serum albumin. Rabbits were immunized with estrone-17-carbamido-human serum albumin and the specificity of the resulting antisera to the estrone residue was established by Schultz-Dale, hemagglutination and turbidimetric techniques employing the principle of hapten-inhibition. The antibody sites complementary to estrone residues were shown to be capable of accommodating also other structurally related molecules. (Supported by grants from the National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Md., and the National Research Council of Canada.)

20 *Immunology*

SPECIFIC ABSORPTION OF THE COMPLEMENT DEPENDENT BACTERICIDAL EFFECT OF NORMAL SERUM. Robert J. Roantree* (intr. by S. Raffel). Stanford Univ. School of Med., Stanford, Calif.

These experiments were undertaken to ascertain whether the serum component in addition to complement necessary for the bactericidal action of normal serum was specific or nonspecific in its action upon any particular bacterial strain. Suspensions of bacteria killed at 62° C were used to absorb fresh individual human sera for 48 hrs at 0°C. Using nine different strains of E. coli and Salmonella in different combinations the results showed that in each case the absorbing strain removed the activity against itself while leaving activity against other strains mostly or entirely intact. It is concluded that with the nine strains tested, the serum component required for the bactericidal action is specific for the particular strain tested.

21 *Immunology*

THE EFFECT OF SPECIFIC ANTIBODIES ON XANTHINE OXIDASE FROM VARIOUS SOURCES. John E. Ultmann and Philip Feigelson (intr. by Sam M. Beiser) Coll. Phys. & Surgs., Columbia University and Francis Delafield Hospital, New York City.

Rabbit antibodies to purified bovine cream xanthine oxidase (X.O.) were prepared. Resultant purified rabbit gamma globulin containing anti-cream xanthine oxidase (Anti-cream X.O.) was absorbed with normal beef serum. Employing the Ouchterlony technic, a reaction of identity was obtained with this Anti-cream X.O. and X.O. preparations from bovine cream, liver, spleen, and kidney. The Anti-cream X.O. yielded no reaction with X.O. of rat liver origin. The velocity of X.O.-Anti-cream X.O. combination was measured turbidometrically. At equivalence, the rate of precipitate formation was the fastest and the catalysis of xanthine by bovine cream X.O. was reduced maximally (60-70% inhibition). Residual enzyme activity was mainly in the precipitate. With increasing X.O. or Anti-cream X.O. concentration, less precipitate formed with disproportionately diminished enzyme inhibition. Neither xanthine, nor 4-amino-pyrazolo(3,4-d)-pyrimidine, prevented the X.O.-Anti-cream X.O. interaction. These data suggest that antibodies are not formed directly to the active center of the enzyme, but that inhibition depends upon steric blockage of the active center by proximal binding of antibodies.

22 *Immunology*

SOME QUANTITATIVE ASPECTS OF AGAR COLUMN DIFFUSION ANALYSES. William G. Glenn (intr. by A. A. Boyden) School of Aviation Med., USAF, Brooks AFB, Texas.

This extensive study on quantitative aspects of agar column immunodiffusion defined the limitations of the technique for homologous human gamma globulin and serum albumin reactions each at 5 antigen and antiserum concentrations. Over 2500 cathetometric measurements on approximately 500 reactions at closely controlled and routine laboratory conditions were done. Statistical analyses showed best over-all accuracy of measurements to be $\pm$ 0.348 mm (approx. 1.5%) ($P < .05$) for both discrete and diffuse zones. A comparison of slopes for penetration vs. time$^{\frac{1}{2}}$ for 300 reactions indicated that antigen concentrations differing by less than 0.1% could not be significantly distinguished; reproducibility of all data was from 68% to 85% and temperature changes of less than 5° C. did not significantly alter the slopes. Relative antiserum concentrations that differed from each other by more than 2.5% could be differentiated by slope analysis at constant antigen concentrations. Interfacial precipitates effected zonal resolution and represented an incomplete or one-dimensional assay.

23 *Immunology*

NATURE OF THE ACTIVE SITE OF ANTI-BENZOATE ANTIBODIES. D. Pressman, G. Radzimski*, A. Shaw* and A. Nisonoff*, Roswell Park Memorial Inst., Buffalo, N.Y.

We have already shown that extensive acetylation of antibenzoate antibody (I) destroys its ability to bind the specific hapten. By acetylation in the presence and absence of hapten it has been shown that the loss of activity is due to an attack on the specific site itself. Low levels of acetylation destroy ability to precipitate with antigen through the nonspecific electrostatic effect of the increased negative charge although binding is unaffected. We have now shown that binding activity lost during acetylation can be partially recovered by exposure of acetylated I to pH 11. This reversibility indicates acetylation of a hydroxy group. Further evidence that the quantitative decrease of binding with increase of pH corresponds to an ionizable group of pK ca 10 and that the site is affected by iodination-implicates tyrosine. The reversibility at pH 11 and the requirement that almost all of the amino groups must be acetylated before binding is affected argues against lysine on the site. Evidence that there is a positive charge in the site thus implicates a quanidinium group. The fact that reversal is not complete at pH 11 (although prolonged exposure of antibody to this pH does not affect binding) may be an indication of heterogeneity of antibody sites with respect to composition.

24 *Immunology*

THE M ANTIGEN COMPOSITION OF GROUP A, TYPE 14, STREPTOCOCCI. Grove G. Wiley. Alfred I. duPont Institute, Wilmington, Del.

The M antigens of Group A streptococci are of special interest among other antigens present because of their close association with virulence. While it is generally thought that one M antigen characterizes each type, the work to be presented shows that the M antigen composition of type 14 streptococci behaves as 2 serologically distinct and specific M antigens, each capable of functioning independently. This was first encountered with a culture of type 14 M+ cocci, which after several subcultures, was found to contain type 14 M+ and type 14 M- cocci. The latter possessed characteristics commonly associated with M+ cocci, multiplying well in normal human blood and being virulent for mice. Precipitin and bactericidal tests with these type 14 M+ and M- cocci revealed that the type 14 M- cocci carried a serologically distinct M antigen which was present also on the type 14 M+ cocci. Tests with other type 14 strains showed that most carried both antigens but that some strains carried one or the other antigen alone. This work shows that the type 14 M antigen composition is more complex than is commonly thought. It is also important in studying the relationship of M antigen to virulence, since it can no longer be assumed, as formerly, that a strain which ceases to react with an anti M serum does not synthesize a secondary M antigen.

25 *Immunology*

DETERMINANTS OF IMMUNOLOGICAL SPECIFICITY IN THE TYPE VI PNEUMOCOCCAL SYSTEM. Paul Rebers*, Esther Hurwitz*, and Michael Heidelberger. Inst. of Microbiology, Rutgers, the State Univ., New Brunswick, N.J.

Inhibition of precipitins was used to appraise the function of carbohydrate-phosphate esters. Partial alkaline hydrolysis of the polysaccharide of type VI, S VI, to multiples of its repeating unit, a tetrasaccharide phosphate, produces the most potent inhibitor in rabbit antiserum, but horse antibodies are not inhibited. Hydrolysis to the single tetrasaccharide phosphate also yields a strongly inhibitory substance, but removal of the phosphate results in a product only one-fourth as effective. The constituent sugars have little effect, and their phosphates are only slightly better. Although two of the four carbohydrate constituents of S VI are attacked by $NaIO_4$, which presumably leaves the phosphate diester linkage intact, the oxidized residue still precipitates more than one-half of the horse antibody. The reaction of S VI in rabbit antiserum is not inhibited by the free sugars, but these effect complete inhibition of certain cross-reactions. Guar gum cross-reacts with horse antibody by virtue of multiple galactose end-groups and this precipitation is more strongly inhibited by galactose than by glucose, mannose, or rhamnose. Quantitative data are submitted.

26 *Immunology*

THE USE OF COMPLEMENT FIXATION FOR STUDY OF POLYSACCHARIDE STRUCTURE. Eleanor Wasserman* and Lawrence Levine. Grad. Dept. of Biochemistry, Brandeis Univ., Waltham, Mass.

The ability of mono- and oligosaccharides of known structure to inhibit precipitin reactions has been used to elucidate the fine structure of blood group substances. (Allen and Kabat, J. Immunol. 82:358, 1959). The large amounts of oligosaccharides needed to inhibit the precipitation of pneumococcus rabbit immune systems limit the use of this technique. A quantitative micro-complement fixation technique has been developed. This technique has been used to study the inhibition of homologous pneumococcus polysaccharide-rabbit pneumococcus antiserum systems by mono- and disaccharides. With the pneumococcus type II rabbit system, 0.5μm of glucuronic acid inhibited 50%, while 8μm melibiose inhibited 15%, and 8μm lactose or galactose were not inhibitory. With the XIV rabbit immune system, 0.6μm lactose inhibited 30%, 8μm melibiose 30%, 8μm galactose 20% and 10μm glucose or glucuronic acid were not inhibitory. Inhibition of rabbit III, VI, XVIII and M61 antigen-antibody reactions by mono- and disaccharides have been studied.

27 *Immunology*

TWO NORMAL HUMAN 7S γ-GLOBULIN ANTIGENS AND THEIR IMMUNOCHEMICAL RELATIONSHIP TO TWO MYELOMA γ-GLOBULINS REVEALED BY RHESUS MONKEY PRECIPITINS TO NORMAL 7S γ-GLOBULINS. Sheldon Dray. Nat'l. Insts. of Health, Bethesda, Md.

Monkeys were selected for the preparation of antibodies to human serum components because antibodies prepared in a closely related species might be more discriminating for minor antigenic differences among the serum proteins than antibodies prepared in more distantly related species. Monkeys immunized with a variety of preparations containing normal human γ-globulins yielded precipitins to many components of human serum. The monkey antisera reacted with normal human sera in immunoelectrophoretic experiments to give two precipitin bands with the 7S γ-globulins. These two bands represent two antigenically different 7S γ-globulins and were found in each of 24 normal human sera tested thus far. In similar experiments with two myeloma sera, each myeloma γ-globulin reacted with the monkey precipitins to yield only one precipitin band. Mixtures of the two myeloma γ-globulins resulted in two precipitin bands, and various mixtures of the myeloma and normal γ-globulins did not result in more than two precipitin bands. Furthermore, immunoelectrophoretic experiments combined with absorption techniques revealed that in their reactions with the monkey antisera, each myeloma γ-globulin appears to correspond antigenically to one of the normal 7S γ-globulins.

28 *Immunology*

MEASUREMENT OF THE PRIMARY BINDING CAPACITY OF ANTIBODIES DIRECTED AGAINST STREPTOCOCCAL M PROTEIN. Howard M. Grey (intr. by Richard S. Farr). Univ. of Pittsburgh School of Medicine.

A streptococcal antigen has been prepared which is soluble in 40% saturated ammonium sulfate (SAS) but becomes precipitable in 40% SAS when bound to specific antibody globulin. An acid extract (J. Exp. Med., 47:92, 1928) of type 12 streptococci was labeled with I^{131} and most of the non-type specific antigen removed by precipitation with 40% SAS in the presence of heterologous antiserum directed against 6 other types of streptococci. This partially purified supernatant (I^*M_{12}) was approximately 10% insoluble in 40% SAS in the presence of normal rabbit serum, 60% insoluble in the presence of homologous-type 12 antiserum, and 20% insoluble in the presence of hyperimmune heterologous-type antiserum. An increased precipitation of I^*M_{12} was still observed when the homologous-type 12 antiserum was diluted as much as 1:7000, whereas the effect was absent when the heterologous-type antiserum was diluted 1:270. In addition to measuring the I^*M_{12} binding capacity of a given antiserum, diversity of antisera directed against type 12 streptococci could be demonstrated by means of I^*M_{12}-antibody dissociation curves and the effect of antigen dilution on binding capacity. It was shown that antibody present early during immunization was more readily dissociated from I^*M_{12} than was antibody present later in the course of immunization.

29 *Immunology*

SIMILARITIES AMONG MAMMALIAN ACUTE PHASE PROTEINS. E. Gotschlich* and C.A. Stetson. N.Y.U. College of Med., New York.

Acute phase sera of rabbits and monkeys have been found to precipitate with sheep antisera to human C-reactive protein. Similarly, sheep antisera prepared against recrystallized rabbit Cx-reactive protein show cross-precipitation reactions with acute phase human and monkey sera and with crystalline human C-reactive protein. Antigenic crossreactivity between the acute phase proteins of man, rabbit and monkey has also been demonstrated by passive cutaneous anaphylaxis and by the occurrence of delayed skin reactions in guinea-pigs sensitized to the heterologous acute phase protein. The antigenic similarity between these proteins may be related to their common capacity to react with pneumococcal C-polysaccharide.

30 *Immunology*

IMMUNOCHEMICAL AND BIOLOGICAL PROPERTIES OF CROSS-REACTING SYSTEMS. W. O. Weigle. Dept. of Path., Univ. of Pittsburgh, School of Med., Pittsburgh, Pa.

Immunological reactions were performed between rabbit anti bovine serum albumin (BSA) and 10 heterologous serum albumins from mammals. All 10 albumins reacted with the anti BSA by precipitating 5-90% of the antibody and forming bands in agar diffusion plates. With the heterologous precipitin reactions, initial combining ratios and combining ratios at equivalence were generally lower than with the homologous reaction. All of the I^{131} activity contained in solutions of I^{131} labeled heterologous albumins was precipitated in the presence of excess antibody. Absorption of anti BSA with one heterologous albumin always removed some antibody that reacted with any other albumin, but this removal was rarely complete. In double diffusion in agar, some degree of identity was always observed between any 2 heterologous albumins. The bands formed in agar with the different heterologous albumins and anti BSA showed identity with 2, 3 or all of the 4 bands obtained between partially degraded BSA (pepsin treated) and anti BSA. When guinea pigs were injected with 25 μg (N) of anti BSA which would react with a given heterologous albumin and challenged 2 days later with the same albumin, anaphylactic reactions were obtained. With some heterologous albumins, the degree of anaphylaxis was comparable to that produced with BSA.

31 *Immunology*

THE IMMUNOLOGIC AND MORPHOLOGIC CONSEQUENCES OF PROLONGED DAILY INJECTION OF BOVINE ALBUMIN (BSA). Frank J. Dixon, William O. Weigle, Jacinto Vazquez, and Joseph D. Feldman. Univ. of Pittsburgh School of Medicine, Pittsburgh, Penna.

Rabbits were injected daily, six times/week with a given dose of BSA varying from 5-100 mg for many months. While antibody responses varied considerably, some of the rabbits receiving 10-50 mg BSA/day achieved serum antibody levels of 2-3 mg Ab.N/ml within 4-6 weeks. These high levels of circulating antibody were maintained for several months. Some of the best antibody producers developed a transitory arteritis and glomerulitis of the serum sickness type during the second and third weeks of immunization. Thereafter, these rabbits with highest levels of circulating antibody showed only occasional anaphylactic or embolic phenomena, a slight thickening of glomerular capillaries and inconstant minimal proteinuria. Those rabbits with moderate antibody responses suffered few if any consequences of antigen injections. The rabbits making barely enough antibody to combine with all the antigen developed a chronic, progressive glomerulonephritis, either proliferative or membranous, associated with uremia, proteinuria and hypercholesterolemia. Those rabbits making no detectable antibody developed no morphologic abnormalities.

XUM

32 *Immunology*

THE DETERMINATION OF ANTIGEN AND ANTIBODY CONCENTRATIONS IN ABSOLUTE WEIGHT UNITS BY MEANS OF THE SERUM AGAR TECHNIQUE OF OUDIN. Elmer L. Becker and Alonza R. Hayden*. Walter Reed Army Institute of Research, Washington, D. C.

In the method to be described one measures the band migration rate and band optical densities for several concentrations of antigen and/or antiserum. In addition, one employs: (1) a linear transformation of the equation of Wales (J. Chimie physique 55:338,1958), (2) a standard curve relating the optical density of a precipitate band to the amount of N precipitated at equivalence/ml of antiserum present, and (3) an empirical relationship between D_1, the diffusion coefficient of the antigen and R', the ratio (in absolute units) of antigen to antibody at equivalence. For each antigen-antibody system, by substituting the migration rate into (1), one finds D_1 and R_o, the ratio (in relative terms) of antigen to antibody giving no movement of the band; the band density and (2) gives the N precipitated at equivalence from 1 ml of antiserum; while from D_1 and (3) one obtains R'. The precipitate N/ml and R' give the antibody N/ml for the given system, while R_o, R' and antibody N/ml are employed to yield the antigen N/ml of antigen solution. The results obtainable with this method will be demonstrated for an impure bovine gamma globulin-rabbit antiglobulin system.

33 *Immunology*

REACTIVITY OF DENATURED ANTIGENS TOWARD ANTISERUM AGAINST THE NATIVE ANTIGEN. Arthur A. Hirata (intr. by Dan H. Campbell). Division of Chemistry and Chemical Engineering, California Institute of Technology, Pasadena.

If the antigen is insoluble, then all the antibody of a given serum should combine and be absorbed within the limits of dissociation of the complex. Attempts were made to simulate the above condition with soluble antigen systems by making the antigen insoluble without seriously affecting the combining sites. The antigen, bovine serum albumin (BSA), was made insoluble by formaldehyde or by heat treatment. Neither one of these denatured BSA's absorbed all the antibody when used alone, i.e., when the denatured antigen was mixed with chicken antiserum prepared against native BSA, some precipitating antibody remained in the supernatant regardless of the amount of denatured antigen added. However, when the antiserum was added to a mixture of formaldehyde and heat denatured BSA, no antibody remained in the supernatant as determined by the interfacial test. Investigation is in progress to adapt this procedure to estimate the total antibody content of antiserums.

34 *Immunology*

INFLUENCE OF TEMPERATURE ON THE SENSITIVITY OF PARACOLOBACTRUM BALLERUP TO THE BACTERICIDAL ACTION OF SERUM. Etsuko Osawa (intr. by L. H. Muschel). Dept. of Serology, Div. of Communicable Dis., Walter Reed Army Inst. of Res., Wash., D. C.

Gram negative bacteria, particularly members of the Enterobacteriaceae, are generally sensitive to the bactericidal action of the antibody-complement system. Exceptions exist and include certain strains of P. ballerup, Salmonella paratyphosa C and Salmonella typhimurium. These organisms have been found to be resistant to rabbit antisera and various mammalian complement sources. It was noted, however, that P. ballerup cultured overnight at temperatures of 40°C and higher, instead of 37°C, became markedly sensitive to the bactericidal action of normal and immune sera. This sensitivity was not transmissible since, on recultivation at 37°C, the organism regained its serum resistance. On the other hand, when S. paratyphosa C was cultivated at 40°C or higher, it remained refractory to the bactericidal action of immune serum, but became very slightly sensitive to normal serum. Moreover, unlike most organisms which are relatively resistant to serum bactericidal action, S. paratyphosa C is O-agglutinable. The findings suggest that a host's temperature may influence its antibody defenses against certain parasites and that serum resistance may involve different mechanisms in different organisms.

35 *Immunology*

ANTIBODY SPECIFICITY OF MAMMALIAN CELLS GROWN IN VITRO. T. T. Puck and M. Oda.* Dept. of Biophysics, Univ. of Colo. Med. Center, Denver.

Colonial growth of single mammalian cells permits quantitative assay of antibodies which prevent cell multiplication. Antisera were prepared by 3 rabbit injections of a suspension containing Freund adjuvant and 10^7 cells from a clonal stock, grown in vitro. Antisera were titrated by adding various dilutions of the antiserum to petri dishes along with the cell inoculum and scoring the colonies developing after incubation. Quantitative demonstration of antibody action is reproduceable in dilutions of 1:500, and occasionally 1:2000. Complement is required for maximum antibody effectiveness, but even in its absence, complete suppression of cell multiplication can be obtained if the antibody concentration is raised to 4%. Antibody inhibition of cell reproduction is irreversible after 1 hour at 37°C. A high degree of species specificity is demonstrable by these methods. Thus, antisera to the S3 cell, a hyperploid cell of human cancer origin, and to diploid Chinese Hamster cells are highly inhibitory to their homologous cells but demonstrate little cross reaction. Antisera to normal human cells originating in various tissues have been prepared, and are being studied for their reaction specificity.

36 *Immunology*

IMMUNOCHEMISTRY OF NORMAL AND GENTICALLY ALTERED TRYPTOPHAN SYNTHETASE FROM NEUROSPORA CRASSA. Michael Carsiotis* and Sigmund R. Suskind. McCollum-Pratt Institute, The Johns Hopkins University, Baltimore, Maryland.

Rabbit antisera have been prepared against highly purified samples of tryptophan synthetase (Tsase) and the antigenically related protein (CRM) from tryptophan mutant td_2. The gamma globulins were exhaustively absorbed with extracts of a mutant which forms neither Tsase nor CRM. These anti-enzyme fractions were tested against homologous and heterologous antigen preparations by both quantitative precipitin and neutralization methods. Supernatant analyses were carried out for enzyme, antigen and anti-enzyme activity. In all cases there was close correspondence between neutralization and precipitin equivalence for both the homologous and heterologous systems. The data obtained are in good agreement with values calculated from the Heidelberger and Kendall equation. They indicate an antibody/antigen N ratio between two and three and a molecular combining ratio of approximately two at equivalence. The enzyme-anti-enzyme precipitate can be dissociated by acid. These data provide further proof of the structural similarity between normal Tsase and the genetically altered td_2-CRM protein.

37 *Immunology*

ANTIBODY RECOVERY AND VIRUS REACTIVATION FOLLOWING DISSOCIATION OF ANTIBODY-VIRUS COMPLEXES. Irving Rappaport. Dept. of Botany University of California, Los Angeles 24, Calif.

Complexes of rabbit antibody and tobacco mosaic virus (TMV) were incubated at 4 degrees centigrade for 24 hours at different pH's, varying from 2.0 to 7.0. The fraction of antibody dissociated at the end of this period of time followed an "S" shaped curve, where essentially all of the antibody was dissociated at pH 2.0. The inflection point occurred at approximately pH 3.5. Although antibody recovery could be considered complete, by this procedure, recovery of infectious virus following such acidification and separation from antibody by centrifugation was less successful. Greater recoveries of virus activity were obtained when specifically purified antibody was used in these experiments. However, in no instance, even with purified antibody, was 100% of the virus infectivity recovered. Another means for demonstrating reactivation of antibody inactivated TMV was through addition of large excesses of ultra-violet inactivated TMV as an antibody competitor. Again, the best evidence for reactivation was obtained when purified antibody rather than whole antiserum was used in the experiments.

38 *Immunology*

QUANTITATIVE STUDIES OF THE INHIBITION OF THE FIBROIN-RABBIT ANTI-FIBROIN SYSTEM. John J. Cebra (intr. by M. W. Chase). The Rockefeller Institute, New York City.

The isolation and characterization of peptides from chymotryptic hydrolysate of silk fibroin and their capacities to inhibit the fibroin-rabbit anti-fibroin precipitin reaction have been reported. After inactivation and removal of chymotrypsin, total hydrolysate of fibroin (average chain length 15-19 amino acids) gives 90-100% inhibition at 120-150 μm N/ml. By use of molecular sieving by Sephadex G-25, a mixture of peptides (average chain length 9-12 amino acids) can be prepared free of high MW peptides. At a concentration of 200 μm N/ml this fraction gives 75% inhibition in one system. The major components of this fraction are tetrapeptides Gly(Gly, Ala)Tyr and Gly(Gly, Val)Tyr (20% inhibition at 18-20 μm/ml), octapeptides $Gly(Gly_3, Ala_2, Val)Tyr$ (30% inhibition at 8-14 μm/ml) and $Gly(Gly_3, Ala_3)Tyr$ (40% inhibition at 5-18 μm/ml), and dodecapeptides containing two Tyr/molecule. These latter give 50% inhibition at 5-8 μm/ml and yield the above octa- and tetrapeptides in equimolar amounts upon hydrolysis by chymotrypsin. The synthetic peptide Gly-L-Tyr gives only 0-10% inhibition at 48 μm/ml. If % inhibition is plotted vs. μm/ml of peptide inhibitor, a straight line results which may reach a plateau depending on the antiserum used. The five systems studied contained 8-16 γ Ag N/ml and 50-70 γ Ab N/ml.

39 *Immunology*

EFFECT OF DIAZONIUM COUPLING ON ANTI-FORSSMAN ANTIBODY. Felix Borek and Arthur M. Silverstein (intr. by S. B. Formal) Armed Forces Inst. of Pathology, Washington, D.C.

Rabbit anti-Forssman serum was coupled with p-diazophenylethanol, p-diazophenylacetic acid, and p-diazophenylsulfonic acid in theoretical amounts from 4 to 58 moles of diazonium salt per mole of protein. The degrees of coupling to protein of similar initial molar ratios of each compound were found to be comparable, as judged by the ratios of u.v. absorption at 280 mμ to total nitrogen. As more groups were coupled to the protein, the hemolytic activity, as determined with sheep cells in the presence of excess complement, was found to decrease. This effect was more pronounced for those diazonium salts carrying an anionic group than was the case for the nonionic alcohol. The effect of treatment on agglutination titers, using the same treated sera and sheep cells, did not parallel the reduction in hemolytic titers; agglutination appeared to be less affected by the chemical treatment. The results suggest that inhibition of serologic activity is in part related to the blocking of tyrosine and/or histidine residues, and support previous demonstrations of the non-specific effect of adding charged groups upon the serologic activity of antibody.

40 *Immunology*

STUDIES ON ANTIGEN-ANTIBODY DIFFUSION IN AGAR PLATES. Frederick Aladjem, Henry Klostergaard* and Rita L. Paldino*. Dept. Med. Microb., Univ. So. Calif., School of Med., Los Angeles, Calif.

To establish experimentally the conditions of precipitation in agar diffusion plates, precise measurements of the time of appearance and of the coordinates of the initial zone of precipitation are essential. The desired accuracy was obtained with the following arrangement: a central cylindrical well, and concentrically, an outer annular well, with proper spacing between them. The zone of precipitation is observed as a ring. Using the assumptions previously stated (Aladjem, et al., J. Immunol. 83: 221, 1959), expressions similar to eqs. 15, op. cit., result when proper limits of integration are applied. If it is assumed that the zone of precipitation cannot be trespassed by either antigen or antibody, the following relation is obtained:

$$C_{(r,t)} = \frac{2C_o}{x_p^2} \int_{s_1}^{s_2} \sum_{n=1}^{\infty} \frac{J_o(r\alpha_n)\, J_o(r'\alpha_n)}{[J_1(\alpha_n x_p)]^2} e^{-D\alpha_n^2 t} r'\, dr'$$

where $C_{(r,t)}$ is the concentration of the diffusing material at a distance r from the center of the center well at time t, C_o is the initial concentration, x_p the distance of the zone of precipitation from the center, s_1 and s_2 the radii of the reservoir, D the diffusion coefficient, J_o and J_1 Bessel functions of the first kind of order zero and one, respectively, α_n the positive roots of $J_o(\alpha x_p)=0$, and r' the variable of integration. (Supported by a grant from USPHS.)

41 *Immunology*

SEROLOGIC STUDIES OF SALMONELLA TYPHOSA. E. E. Baker and E. E. Whiteside. Boston Univ. School of Med., Boston, Mass.

The antigenic structure of Salmonella typhosa has been studied by means of the Ouchterlony technic. The antigens employed were aqueous extracts of whole acetone-killed bacilli. Antigens of S. typhosa strain Ty2 tested against homologous serum formed five zones of precipitate. Ty2 antigens tested with S. schottmulleri serum (XII antibody) gave two lines and with S. haarlem serum (IX antibody) gave one line. Ty2 serum tested with extracts of Shigella flexneri Type 3 gave three lines. Since these antigen-antibody systems probably were not related to the IX and XII systems, the Ty2 serum was absorbed with S. flexneri Type 3 organism after which it still produced five lines with Ty2 extracts. This finding indicated that more than five antigens are present in S. typhosa. Ty2 antigen was absorbed in agar plates with either haarlem or schottmulleri antibody and tested with Ty2 serum absorbed with flexner bacilli and Vi antigen. All of the remaining Ty2 antigens were absorbed by schottmulleri serum. Thus, if the IX and XII antigens are associated on the same molecule there must be other antigens in common between two organisms. In contrast, the haarlem serum only absorbed one of the antigens in the Ty2 extracts but the data are insufficient to determine whether or not both IX and XII antigens were removed.

139. IMMUNE RESPONSE-Experimental Disease

1 *Biochemistry*

HOST INFLUENCE ON PSITTACOSIS GROWTH. Jack Litwin (intr. by J. W. Moulder) Dept. Microbiol., University of Chicago, Chicago, Ill.

The 6BC strain of psittacosis growing in Chang's liver cells or the chick embryo chorio-allantoic ectoderm (CAM) showed similar growth characteristics and morphology with the light microscope. However, when thin sections were examined in the electron microscope an important difference was observed. The 6BC strain growing on the CAM showed a mixed population of dense-centered and reticulated particles resembling feline pneumonitis virus (J. Inf. Dis., 105:129, 1959). Most of the dense-centered particles were formed about 30 hours after infection; they never had more than one dense-center. In Chang's liver cells, however, dense-centered particles were formed before 25 hours and particles with more than one dense-center were frequently observed. The percentage of dense-centered particles seen within Chang's cells was greater than the percentage within CAM cells at comparable times in the growth cycle. These observations suggest that different host cells may greatly influence the growth of this micro-organism and its cellular pathogenicity which may be inversely related to the percentage of dense-centered particles produced within the vesicle. (Aided by grant No. E-1594 from the Nat'l Inst. Allergy and Infectious Diseases, PHS).

2 *Biochemistry*

FRACTIONATION OF GUINEA PIG BRAIN PROTEINS WITH ENCEPHALITOGENIC ACTIVITY. M.W. Kies, J.B. Murphy* and E.C. Alvord, Jr.* Natl. Insts. of Health, Bethesda, Md. and Baylor Med. School, Houston, Texas.

The highly encephalitogenic guinea pig brain protein previously described (Kies, M.W. and Alvord, E. C., Jr., Chapt. 12 of "Allergic Encephalomyelitis" Publ. by C.C. Thomas, 1959) has been further fractionated on modified cellulose columns. Two fractions were obtained, only one of which was encephalitogenic in guinea pigs. The active fraction (58% by weight of the dialyzed water-soluble protein) is not adsorbed on DEAE cellulose from water solution, but is retained completely on a CM cellulose column of similar proportions (10 mg. cellulose/mg. protein). The encephalitogenic fraction (DEAE eluate) contains 18% N, 0.68% hexose and <0.1% hexosamine. In the ultracentrifuge it exhibits one symmetrical peak with an $S_{20,w}$ of 0.9S (μ = 0.1, pH 4). The inactive (CM eluate) fraction contains 14% N, 2% hexose and 0.3% hexosamine. Two major peaks were observed on ultracentrifugation ($S_{20,w}$ = 1.6S and 9.7S). The isoelectric point of the DEAE cellulose eluate is 10.4; that of the CM cellulose eluate approximately 7 (by turbidity measurements).

XUM

3 *Pharmacology*

MENGO VIRUS INFECTION IN BACILLUS CALMETTE-GUÉRIN OR ZYMOSAN TREATED MICE. L.J. Old*, D.A. Clarke, and B. Benacerraf.* Div. Exp. Chemo. Sloan-Kettering Institute, S-K Division Cornell University Medical College, New York, N.Y. and New York University College of Medicine, New York, N.Y.

The course of infection with Mengo virus was investigated in Swiss mice previously treated with zymosan or B.C.G., agents known to influence the functional activity of the reticuloendothelial system, increase capacity to respond to antigenic stimulation and enhance resistance to bacterial and certain transplanted tumor challenge. The infection was induced by intraperitoneal inoculation of serial dilutions of a stock preparation from infected brain homogenate. The LD_{50} in control animals in five separate experiments ranged from 7.3 to 8.0 (-log dilution). Animals challenged with virus at 12, 19, 21 or 26 days following inoculation of 1 mg. B.C.G. (wet weight) were less susceptible to virus lethality as demonstrated by increased survival time and a LD_{50} of 6.0 to 6.75. This protective effect was still apparent 3.8 months after B.C.G. A similar degree of resistance to Mengo virus (average LD_{50} 6.55) was achieved by prior treatment with 1 or 2 intravenous injections of 1 mg. zymosan.

4 *Pathology*

PATHOGENESIS AND PATHOLOGY OF "RUNT" DISEASE IN RATS. V. Defendi, D. Steinmuller, R. E. Billingham and W. K. Silvers (intr. by D. R. Coman.) Wistar Institute, Philadelphia, Pa.

Cell suspension of lymphatic tissue from adult rats of one inbred strain (Lewis) inoculated intravenously in newborn rat of another inbred strain (BN) produce a characteristic syndrome in the recipient animals, described as "runt" disease. With appropriate doses of lymphoid cells the disease is fatal within 15-20 days. The sequence of the clinical and pathological changes has been followed in several groups of animals. In the terminal stage the animals are grossly underweight, hypothermic, with diarrhea, exfoliation, and thickening of the skin. There is complete atrophy of the thymus and enlargement (3 to 5 times normal) of spleen and lymph nodes; this enlargement is the result of proliferation of histiocytes and reticulocytes with almost complete disappearance of mature lymphocytes. The blood and bone marrow reflect these changes with the picture of hypoplastic anemia and absolute keukocytosis with inversion of the lymphocytes/granulocytes quotient. The general expression of the disease is compatible with the hypothesis of an immunological reaction of competent foreign cells toward an immature host. The peculiar manifestations, not described in other types of "runt" or "wasting types of disease, are possibly related to the different species and to the physiological condition of the host.

5 *Pathology*

PRODUCTION OF A "MONGOLIAN-IDIOT"LIKE ABNORMALITY IN HAMSTERS. Helene Toolan. Sloan-Kettering Inst.for Canc.Res.,New York,N.Y.

Over 5000 hamsters were treated in utero, at birth, and various periods after birth with fractions of fresh and frozen transplantable and spontaneous human and rat tumor cells, and normal human, rat, and hamster tissues. A specific abnormality characterized by small size, flat faces, protruding eyes and tongue, abnormal or absent teeth, and fragile bone structure was produced in the animals treated at birth and often in utero with cell-free filtrates and certain of the cell fractions from six different transplantable human tumors. Results were uniform and unvarying according to the material used. A few such animals were produced by injecting fractions of spontaneous human cancers or the livers of tumor donors. One was noted after treatment with spleen from a rat carrying a spontaneous tumor. None were seen after treatment with hamster tissues or rat tumors. No tumors have been found in 400 of the effected animals although many are over a year old. The animals are fertile and, bred to one another, produce normal offspring. No effect has been produced in baby mice similarly treated. The active materials are very stable but can be rendered ineffective by small amounts of normal serum and, in most instances, by heating. Electron microscope studies have yielded to date no virus agent. The possible nature of the effective factor(s) will be discussed.

6 *Pathology*

AN IMMUNO-PATHOLOGIC STUDY OF AVIAN NEPHROTOXIC NEPHRITIS IN THE RABBIT. L.R. Levy*, J.B. Sawyer* and I.H. Lepow, Inst. of Path., Western Reserve Univ., Cleveland.

Masugi, Kay, Lange and others have produced glomerulonephritis in rabbits by injection of anti-rabbit kidney duck serum(ARbKDS). The present study was designed to correlate in one experiment previously studied immunopathologic changes in this disease with alterations of a recently described serum inhibitor(EI) of an enzyme derived from the first component of complement(C'1-esterase). One group of rabbits was given a single IV injection of ARbKDS; another group received the same dose of normal duck serum; and a third served as uninjected controls. 24 hr.-urinalysis, serum antibodies to duck serum, presence of circulating duck serum, serum complement and EI (Levy and Lepow, PSEBM 101:608, 1959) were measured daily for 13 days. One ARbKDS-rabbit was autopsied daily and control rabbits at wider intervals. Serum chemistries were obtained prior to autopsy. Close correlations existed among time of onset(4-5 days) of clinical and histopathologic disease, appearance of antibodies to duck serum, and transient but significant decreases of titer of complement and EI in rabbits receiving ARbKDS.

7 *Pathology*

PREVENTION OF EXPERIMENTAL ALLERGIC ENCEPHALOMYELITIS WITH 6-MERCAPTOPURINE AND NITROGEN MUSTARD. L. Hoyer*, R.A. Good and R.M. Condie*. University of Minnesota, Minneapolis.

Biweekly injections of HN_2 prevented development of EAE. The effect was not associated with marked leukopenia. Large doses of HN_2 given nine days following injection of spinal cord-adjuvant delayed appearance of EAE. This effect was apparently due to the action of HN_2 on circulating leukocytes. Schwartz inhibited antibody production to BSA with 6-Mercaptopurine in rabbits so the effect of this drug on EAE was studied. 6-Mercaptopurine completely suppressed the appearance of EAE in rabbits during the period in which an adequate dosage of the drug was given. After termination of treatment, animals developed EAE after a latent period of 7-10 days. Smaller dosages of 6-Mercaptopurine delayed appearance, but did not completely prevent EAE. Guinea pigs in whom EAE was prevented by 6-Mercaptopurine did not develop EAE following discontinuation of the drug. 6-Mercaptopurine did not completely inhibit formation of antibody when BSA was administered in Freund's adjuvant. The apparent contradiction between this finding and Schwartz's observations is believed to be due to the more intense stimulation provided by BSA in adjuvants. (Aided by grants from U.S.P.H.S., Minnesota Heart Assoc. and American Heart Assoc.)

8 *Pathology*

GAMMA GLOBULIN STUDIES IN EXPERIMENTAL ARTERITIS. S. Cohen, O. Ohta, E.J. Singer & L. Strauss (intr. by H. Popper). Dept. of Pathology, Mt. Sinai Hospital, New York, N.Y.

Arteritis was produced in rats by unilateral nephrectomy followed by Doca administration or silk wrapping of the contralateral kidney and in rabbits by horse serum injection. Vascular lesions were studied by histological and Coons techniques. In the rat gamma globulin (GG) fluorescence was observed in the necrotic media of small arteries. GG was absent from undamaged vessels in both species. It was found inconsistently in intimal proliferation and perivascular infiltration. In rabbits variable amounts of intracellular GG were found in granulomatous tissue replacing the vessel wall after acute arteritis. Upon healing by fibrosis no GG was present. Some sections showing GG in vessel walls were treated with pH 3.4 buffered saline for 2 hours before using fluoresceinated anti-GG. Significant reduction of fluorescence was observed almost exclusively in rabbit lesions indicating probable elution of the GG. Controls treated with pH 7 buffered saline showed no change in fluorescence. The results of elution experiments are consistent with the hypothesis that the GG in hypersensitivity angiitis is part of an antigen-antibody complex in the vessel wall. In necrotizing arteritis of rats the GG, which behaves differently, is probably not antibody. (Supported by a grant from the Block Foundation).

9 *Immunology*

EXPERIMENTAL POLIOMYELITIS FOLLOWING INTRAMUSCULAR VIRUS INJECTION. Neal Nathanson (intr. by D. Bodian.) Dept. of Anatomy, Johns Hopkins U., Balt., Md.

Human poliomyelitis cases, following injection of virulent virus, were frequently paralytic, with a marked tendency for localization of first paralysis in the injected limb. Two alternative mechanisms were postulated. Neural spread: virus enters the peripheral nerve from injected muscle, and travels via nerve to the corresponding cord segments. Viremia with provoking effect: virus enters the blood and penetrates the blood-brain barrier, which is more permeable in the area innervating the injected muscle. These mechanisms were tested in cynomolgus monkeys. The MV strain caused paralysis with a high frequency of localization. Nerve block conferred complete protection, while passive antibody had no effect (neural spread operative). The Mahoney strain caused paralysis, with localization less frequent. Passive antibody protected, while nerve block only enhanced localization (dominance of viremia and the provoking effect). (Supported by a grant from The National Foundation, Inc.)

10 *Immunology*

VALIDITY OF 50 PER CENT ENDPOINTS FOR COMPLEMENT FIXATION PROCEDURES. Stanley Marcus and William Wu*, Dept. of Bact., College of Medicine, Univ. of Utah, Salt Lake City.

It is customary to employ so-called 100 per cent hemolysis as the endpoint in C' titrations particularly in diagnostic C' fixation tests. The relative ease with which C' concentrations, in a defined system, may be determined spectrophotometrically, plus certain assumptions, has led to the description of C' fixation tests for routine use in which C'50 endpoints are employed. The theoretical basis for employing such methods is clear, that is, reproducibility of the endpoint. However, where data are available for comparing results of antibody estimations obtained by both systems, it is evident that neither is superior. As a result of tests to be described it was learned that the so-called "100 %" C' estimation method is not quantal; that neither visual nor spectrophotometric procedures detect nonlysed cells as the asymptote is approached. The following hypotheses will be supported: the "100 %" method is highly reproducible; qualitative tests to detect antibody by this method are sensitive and specific. (Supported by Grant No. E-2269, Nat. Inst. All. & Inf. Dis.)

11 *Immunology*

LUNG LOCALIZING ANTIBODIES. Y. Yagi, I. Tamanoi* and D. Pressman, Roswell Park Memorial Inst., Buffalo, N.Y.

The rate of fixation in the rat of lung localizing antibodies was determined by radioiodinating specifically purified lung localizing antibody and injecting it into donor rats which were bled at various times. The blood was assayed for remaining lung localizing activity by injecting it into recipient rats and determining the radioactivity fixed in the lung. The fixation was very rapid and essentially all localizing activity was removed within 3-6 minutes or essentially as the blood flowed through the lungs.

The lung localizing antibody was prepared by injecting rabbits with rat lung tissue and was concentrated by adsorbing it on lung "mitochondria" and then eluting it. The mitochondrial fraction (Hogeboom) was more effective for purification than the nuclear or microsomal fractions or the soluble part fixed on diazotized aminopolystyrene. Kidney localizing antibody present in antiserum prepared against lung was removed by treatment with the readily sedimented components of kidney homogenate.

Supported by a grant from the USPHS.

12 *Immunology*

THE INFLUENCE OF X-IRRADIATION UPON THE ACQUISITION OF IMMUNITY TO INFECTIONS. Sidney Raffel, Marjorie LaSalle*, and Ivan Kochan*. Stanford Univ. School of Med., Stanford, Calif.

Sublethal doses of irradiation or lethal doses following AET have been used to determine whether immunity may be acquired to some infectious agents when antibodies are suppressed. AET was found to protect mice against irradiation death while not protecting the antibody-synthesizing mechanism. Two diseases were investigated: poliomyelitis type 2 in mice, and tuberculosis in mice and guinea pigs. Appropriate vaccination was carried out 24 hrs after irradiation (500 or 700 r with AET in mice, 300 to 400 r in guinea pigs). In mice suppression of the antibody response was synonymous with failure to acquire immunity to both agents. Preliminary experiments in guinea pigs, however, reveal a good level of resistance despite doses of x-radiation which completely suppressed antibodies, but not a serum tuberculostatic factor recently described (Kochan & Raffel, J. Immunol., in press).

13 *Immunology*

A NON-SUPPURATIVE NECROSIS OF RETROPERITONEAL FAT IN GUINEA PIGS OCCURRING AFTER INJECTION OF TRYPANOSOMES IN ADJUVANTS. Murray M. Lipton and Alex J. Steigman. Ky. Child Health Fdn. Lab., Univ. of Louisville School of Medicine, Louisville, Ky.

The intradermal injection of a group of guinea pigs with 0.2 ml (0.1 ml/site) of Freund adjuvants containing frozen and thawed Trypanosoma equiperdum (dried at room temperature) and guinea pig spinal cord led to the following findings on termination of the experiment at 23 to 27 days: In 4 of the 5 animals the retroperitoneal fat demonstrated many discrete, pale, opaque, focally hemorrhagic, moderately firm nodules from 5 to 25 mm in diameter. Histological examination revealed irregular diffuse granulomatous areas of fat necrosis including many fat-containing macrophages and giant cells of both Langhans and foreign body type. There were adjacent regions of fresh and recent hemorrhage and of fibroblastic proliferation. Arteritis was not seen. Normal animals from the same shipment and housed in adjacent cages were free of these lesions. The CNS of some of the injected animals had lesions of allergic meningoencephalitis primarily lymphocytic perivascular cuffing. The histological resemblance of these fat lesions to the panniculitis described by Weber and by Christian is of interest as a model to elucidate the etiology of Weber-Christian disease. (Supported by a grant from the National Institute of Neurological Diseases and Blindness.)

14 *Immunology*

AUTOANTIBODIES TO THYROID TISSUE SEDIMENT. Joseph H. Kite, Jr.* and Noel R. Rose, Department of Bacteriology and Immunology, University of Buffalo School of Medicine, Buffalo, N.Y.

The anti-globulin consumption test (AGCT) has been adapted to the demonstration of autoantibodies in the serum of patients with chronic thyroiditis. Normal human thyroid tissue was homogenized and washed 15 times with buffered saline so that the supernatant was free of detectable thyroglobulin and serum. The lyophilized tissue sediment was then used in the AGCT. Positive reactions were obtained with 17 of 43 human thyroiditis sera. No reaction occurred with normal human sera. The results of the consumption tests were compared with hemagglutination tests employing tanned red blood cells coated with soluble human thyroid extract. A direct correlation between the two titers was seen in most but not all cases. The reactions observed in the AGCT appeared specific for thyroid sediment, since other tissue sediments gave no consumption with thyroiditis sera. Absorption of thyroiditis serum with purified human thyroglobulin removed part of the consumption titer. This suggested that some of the thyroglobulin was either tightly bound to the insoluble tissue components or was present in an insoluble form. It also points to the possible presence of antibodies to other insoluble antigens of the thyroid tissue sediment. (Supported in part by research grant C 2357 from N.I.H.)

XUM

15 *Immunology*

IMMUNOLOGIC NATURE OF ARTHRITIS INDUCED IN RATS BY MYCOBACTERIAL ADJUVANT. Byron H. Waksman and John T. Sharp*. Harvard Medical School and Mass. Gen. Hosp., Boston, Mass.

Inflammatory lesions affecting joints, subcutaneous tissues, and uves are produced in rats by a single injection of mycobacteria in oil (Pearson). The following evidence as to pathogenesis is presented: Mycobacteria in oil are necessary and sufficient. The intradermal and foot-pad routes of inoculation are more effective than other routes. There is a latent period of 10-16 days. Reinoculation results in recurrence with a shortened latent period. 600r whole body irradiation before inoculation suppresses the response. Very immature animals are not susceptible. Disease occurs earliest and most intensely at sites of non-specific inflammation. Rats with disease are intensely sensitive to tuberculin. Parenteral injection of tuberculin during convalescence causes a mild flare-up of disease. Injection with tubercule bacilli at birth or repeatedly in the adult inhibits the later production of disease with complete adjuvant. Passive transfer of apparently typical arthritis is possible with the use of sensitized lymph node cells and tuberculin. It is concluded that the disease is probably a disseminated hypersensitive reaction of the delayed type to tubercle bacillus antigen.

16 *Immunology*

PSEUDOMONAS PSEUDOMALLEI AS AN ADJUVANT IN THE PRODUCTION OF ALLERGIC ENCEPHALOMYELITIS. Alex J. Steigman and Murray M. Lipton, Kentucky Child Health Foundation Laboratory, Univ. of Louisville School of Medicine, Louisville 2, Kentucky.

Studies to elucidate the role of killed mycobacteria in the rapid production of allergic encephalomyelitis has led to the search for a substitute for the mycobacteria. Freund immunologic adjuvants were prepared using dried killed Pseudomonas pseudomallei (strain B-1110)and either petroleum ether extract of whole bovine spinal cord or guinea pig spinal cord suspension. Hartley strain guinea pigs were injected contemporaneously in 5 intradermal sites or in 1 site. Symptoms of allergic encephalomyelitis were observed in animals so injected. Symptoms were severe when 0.5 ml of emulsion containing 2.5 mg of pseudomallei organisms and 1 mg of cord extract were injected. Histological examination of the CNS of these animals revealed lesions characteristic of the experimental disease. Injection of the emulsion sine cord extract was without effect. The injection of emulsion containing 0.25 mg of pseudomallei and 4 mg of guinea pig spinal cord (0.1 ml into 1 site) was less effective than with mycobacteria both in severity and frequency of symptoms. Lesions were present in the brain and spinal cord of these animals. Pseudomonas pseudomallei has been found to be an effective adjuvant in the production of allergic encephalomyelitis. (Supported by a grant from the National Institute of Neurological Diseases and Blindness.)

17 *Immunology*

NON-MONOTONIC RESPONSE PATTERNS IN ANIMALS INJECTED WITH WEAKLY VIRULANT CULTURES OF PASTEURELLA TULARENSIS. V. J. Cabelli, L. K. May and A. P. Dufour (intr. by S. Marcus.) Dugway Proving Ground, Dugway, Utah.

It has been observed with most organisms that as the dose is increased the response, as measured by death of the host or by other criteria, also increases. In the studies described it was noted that the response in animals intraperitoneally injected with increasingly greater numbers of organisms was non-monotonic, that in certain dosage ranges there was a decrease in mortality with an increase in dose and that several LD_{50} values were obtained. The above was observed with the weakly virulent Jap 4 strain of P. tularensis and with a culture of the highly virulent Schu strain whose virulence had decreased as a consequence of prolonged storage. Serological results and rechallenge experiments performed on the 12th day following initial injection indicated that the surviving animals in the trough of the response curve had moderate levels of agglutinating antibodies but that they were no more resistant to rechallenge with 5 LD_{50} doses of the virulent Schu strain than were the controls. The interaction of highly and weakly virulent members of the population in the host is suggested as a possible explanation for the aberrant response.

18 *Immunology*

ADRENAL AUTOANTIBODY PRODUCTION IN GUINEA PIGS. Ernest Witebsky and Felix Milgrom*, Univ. of Buffalo School of Med., Buffalo, N. Y.

Guinea pigs were injected with pooled guinea pig adrenal suspension incorporated in Freund adjuvants. Four to ten i.c. inoculations were administered mostly at weekly intervals. The majority of animals produced antibodies against saline extracts of guinea pig adrenals, demonstrable by complement fixation and tanned cell hemagglutination tests. These reactions were both species and organ specific. Extracts of the antibody producer's own adrenals were as active as the pooled adrenal extracts. The active antigenic constituent was thermolabile, possibly of globulin character. In some but not all injected guinea pigs distinct histopathological lesions in adrenal glands were observed. (Supported by grants from N.I.H. and The National Foundation)

140. IMMUNE RESPONSE–Hypersensitivity

1 *Physiology*

INDUCED HISTIDINE DECARBOXYLASE AND B. PERTUSSIS SENSITIZATION OF MICE. R. W. Schayer and O. H. Ganley*. Merck Institute for Therapeutic Research, Rahway, New Jersey.

Injection of mice with Bordetella pertussis vaccine renders certain strains sensitive to histamine, serotonin and anaphylactic shock. In TF mice sensitivity to passive anaphylaxis reaches a maximum on the 3rd or 4th day and persists for 8 to 10 days; lung histidine decarboxylase activity increased after pertussis vaccination reaching a maximum (4 to 8 times normal) on the 3rd or 4th day; activity remained elevated for about 8 days. The period of sensitization coincides closely with the period of increased histidine decarboxylase activity. A number of other vaccines did not show this effect. The hypothesis is proposed that pertussis sensitization is mainly due to a sustained increase in histamine synthesis in lung without concomitant increase in adrenal activity. Prolonged exposure of certain cells to newly-synthesized histamine may produce a reversible alteration in permeability which can be rendered irreversible and lethal by an insult such as injection or release of histamine and serotonin. Ingested adrenal steroids, present after pertussis injection but absent from the body at time of challenge, should block the permeability changes due to histamine and prevent sensitization; this was found to be true. Other evidence supporting the hypothesis and a discussion of its significance will be presented.

2 *Pharmacology*

ANTIHISTAMINIC ACTIVITY OF THE D- AND L-ISOMERS OF DIMETHPYRINDENE (SU-6518). W. E. Barrett, R. Rutledge*, Ann Deitrich* and A. J. Plummer. Research Dept., CIBA Pharmaceutical Products Inc. Summit, N. J.

Recently, dimethpyrindene (Su-6518) was reported to be a very potent, orally effective antihistaminic drug. Dimethpyrindene is a racemic mixture and recent chemical investigations have succeeded in isolating the l- and d-isomers of dimethpyrindene. Both the d- and the l-isomers have been studied in the intact guinea pig to determine their oral antihistaminic activity. The isomers as the maleate salts were administered orally by intubation one hour prior to challenging the guinea pigs with a lethal intravenous dose of histamine. Various doses of the isomers were studied in order that dose response curves might be obtained. From these experimental results it was possible to calculate an ED_{50} value and its standard error for this one-hour time interval. The results obtained were as follows:

ED_{50}-oral dose kg $\pm$ S. E.

L-isomer (Su-8121) - 43 $\gamma \pm 5\ \gamma$
D-isomer (Su-8153) - 13.2 mg $\pm$ 2.13 mg
Su-6518(dimethpyrindene) - 60 $\gamma \pm 10\ \gamma$

Thus in this series of antihistaminic drugs the antihistaminic activity is to be found chiefly in the l-isomer.

3 *Immunology*

TOXIC AND ALLERGIC PROPERTIES OF POLLEN EXTRACTS IN THE MOUSE. Leon S. Kind and Luise Roesner, Univ. of Calif. Med. School, San Francisco, Calif.

In view of recent reports of points of resemblance between endotoxin reactions and hypersensitive responses, we have attempted to induce toxic and allergic reactions in mice with pollen extracts. It was found that water soluble extracts of rye grass (WSRy), timothy grass (WST), bermuda grass (WSB), or ragweed (WSRg) would kill pertussis-inoculated mice in doses which were not lethal to normal animals. In addition, mice sensitized with an alum precipitated whole rye grass pollen complex (APRy) prepared by the method of Fuchs and Strauss (J. Allergy 30, 66, 1959) succumbed to fatal anaphylaxis when challenged with the homologous pollen (WSRy) or related pollens (WST, WSB). Local allergic reactions in the mouse lip were also obtained in animals sensitized with APRy and challenged with WSRy, WST, WSB or WSRg. A laboratory animal is thus available for studying possible in vivo interactions between toxic and allergenic components of pollens. (Supported by a grant from the National Institutes of Health.)

4 *Immunology*

GENERALIZED SHWARTZMAN-LIKE REACTION FOLLOWING S.marcescens SEPTICEMIA IN A FATAL BURN. C.D. Graber, W.T. Tumbusch and E.H.Vogel, Jr. US Army Surgical Research Unit, Ft. Sam Houston, Tex.

Serratia marcescens, an erstwhile pathogen for man (endocarditis, pneumonia), possesses a polysaccharide commonly employed experimentally to induce the generalized Shwartzman reaction in animals. This report describes the microscopic lung, kidney and vascular lesions in a burned patient dying with Serratia septicemia identical to the experimental generalized Shwartzman lesion in rabbits. Bacteriologic and immunologic fabric for the assumption that Serratia functioned as sensitizing and possibly the evoking agent is as follows: The patient experienced a short fulminating course with three blood, two urine and two eschar positive cultures for this organism. Terminal serum had hemagglutinating antibody in titer of 1:32 for the somatic Serratia antigen. Rabbits sensitized with the organism's polysaccharide and evoked with the same material, crude burn diffusate, gamma globulin or dextran sulfate proved Shwartzman competent topically. A fatal generalized reaction in the kidney produced in the same animals was characterized by depositions of fibrinoid material in the glomerular capillary tufts. Altered Properdin and Lysozyme levels in patient also suggested Serratia endotoxemia that could sustain a generalized Shwartzman. The authors are not aware of a previous report of S. marcescens septicemia complicating a burn with resultant generalized Shwartzman syndrome.

5 *Immunology*

SKIN REACTIONS AND IN VITRO LEUKOCYTOLYSIS WITH FRACTIONS FROM CULTURE FILTRATE OF M. TUBERCULOSIS IN GUINEA PIGS SENSITIZED WITH HEAT-KILLED TUBERCLE BACILLI. Bernard W. Janicki (intr. by A. M. Griffin) VA Hospital, Washington, D.C.

Ethanol fractionation of unheated culture filtrate of tubercle bacilli (H37Rv) yielded 3 protein and 2 polysaccharide fractions. Skin reactions produced in sensitized animals by the proteins and the Polysaccharide II fraction seemed to be of the delayed type. The B protein was the most active, followed by C protein, A protein, and Polysaccharide II fractions in order of decreasing activity. Polysaccharide I evoked an immediate-type reaction in some sensitized animals. The gross features of this reaction suggest that it may be an Arthus-type response. Leukocytolysis was measured by a method based on the in vitro release of a lysozyme-like enzyme from peripheral blood leukocytes. Cytolysis tests performed with the fractions showed that Polysaccharide I was the most active. The A protein also was active. The other fractions were more active with sensitive than with normal leukocytes but the degree of specific activity of these fractions was less than that of the Polysaccharide I and A protein fractions. It seems possible that earlier studies of the cytolysis phenomenon employing OT or PPD as the antigen may have measured the effect of either Polysaccharide I or A protein or a combination of both fractions.

6 *Immunology*

PRODUCTION AND TRANSFER OF DELAYED HYPERSENSITIVITY TO MODIFIED HUMAN SERUM PROTEINS IN HUMANS. Paul H. Maurer. Dept. of Pathology, Univ. of Pittsburgh Med. School, Pittsburgh, Pa.

Volunteers were injected intramuscularly several times with solutions of ethylene oxide treated human serum to test the antigenicity of the preparation. During the immunization, several "flare up" reactions occurred at the initial skin test site. When skin tested two weeks after the last injection, the volunteers exhibited reactions of the delayed type. Histological examination of biopsies of the reaction site revealed an almost pure monocyte infiltration without vessel damage or necrosis. Periodic skin testing for a year of the two best reactors revealed the same reaction pattern. Sera obtained from several bleedings failed to reveal antibody detectable by techniques of in vitro precipitation, agar diffusion, and passive systemic and cutaneous anaphylaxis in guinea pigs. In confirmation of the work of Lawrence with bacterial proteins, the delayed hypersensitivity was transferable to normal volunteers injected with the buffy coat of plasma from the sensitized donors. The transfer was successful with live or dead (frozen and thawed 7 x) cells. Histological sections of biopsies obtained from the delayed reaction sites of the recipients will be presented. Immunochemical studies with rabbit antisera produced against the modified human serum indicated the presence of proteins not present in normal human serum.

7 *Immunology*

CHROMATOGRAPHIC FRACTIONATION OF SERA FROM RAGWEED-SENSITIVE INDIVIDUALS. A.H. Sehon, L. Perelmutter* and S.O. Freedman*, Dept. Chem., McGill Univ. and McGill Univ. Clinic, Montreal General Hosp., Montreal, Canada.

Previous investigations demonstrated that skin-sensitizing antibodies could not be separated from the B.D.B.-hemagglutinating factors in allergic sera by different fractionation procedures (Sehon, A.H., Proc. 3rd Internat. Congr. Allergol., Paris, 1958). It was concluded that these two factors may be attributed to the same molecule or that they could be independent entities with similar physico-chemical properties. To elucidate further the nature of these two factors, these sera were fractionated chromatographically on DEAE-cellulose. All the fractions were tested by P-K and hemagglutination methods. Both activities were found associated with each other in the different fractions, but no simple relationship could be established between skin-sensitizing and hemagglutinating titers in any of the fractions. From these findings it appears that under the conditions used for chromatographic fractionation these two activities behave as different serum moieties. However, ultracentrifugal results do not exclude the possibility that these factors are complexed together in the whole serum. (Supported by grants from National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Md. and from the National Research Council, Ottawa).

8 *Immunology*

PREPARATION OF PURIFIED RAGWEED ALLERGENS. D. J. Lea (introduced by A. H. Sehon), Department of Chemistry, McGill University, Montreal, Canada.

In continuation of earlier studies (Sehon, A.H. and Richter, M., J. Immunol. 82, 190, 1959), the water-soluble ragweed extract was depleted of proteins and dialyzed to remove constituents of lower molecular weight. The lyophilized material was fractionated by paper electrophoresis in borate buffers of pH 8.6 and 5.2. At pH 8.6 the yellow pigment and major ninhydrin stainable band (with mobilities corresponding to the fraction previously described as AA_1-D) were associated. At pH 5.2, however, the pigment remained at the point of application, while two bands of ninhydrin stainable material migrated towards the cathode. The chemical composition of these fractions is being established and the allergenicity of various fractions is being evaluated by passive transfer tests and by hemagglutination using the bis-diazotized-benzidine method. The relationship between carbohydrate content (arabinose and galactose being present in non-dialyzable ragweed preparations) and allergenicity is also being investigated. (Supported by grants from the National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Md. and the National Research Council of Canada).

9 *Immunology*

RAPID INDUCTION OF ALLERGIC ENCEPHALOMYELITIS IN RATS WITHOUT THE USE OF MYCOBACTERIA.
Jennifer Bell* and Philip Y. Paterson. N.Y.U. Coll. of Med., New York and National Institutes of Health, Bethesda.
Other workers have reported the necessity of combining nervous tissue inocula with Freund's complete adjuvant (emulsifying agent, paraffin oil and killed mycobacteria) for rapid, regular induction of allergic encephalomyelitis (AE) in guinea pigs, rabbits and monkeys. Forty-eight adult rats received one intracutaneous injection of guinea pig spinal cord combined with Freund's incomplete adjuvant, i.e. without the mycobacteria. Sixteen rats had hindleg paralysis within 12-18 days. AE lesions were found in these 16 as well as in 26 of the remaining 32 rats (killed 22-26 days post-injection). In contrast, AE lesions were present in only 2 of 16 additional rats which received the spinal cord antigen alone. In the rat, emulsifying agent-paraffin oil, without addition of mycobacteria, provide the necessary adjuvant effect for rapid, regular induction of AE.

10 *Immunology*

DETECTION OF ANTIBODIES DIRECTED AGAINST SALICYLATES.
Lawrence M. Weiner and Homer Howes. (intr. by M. Goodman.) Depts. of Microbiology and Medicine, Wayne State Univ. Coll. of Medicine, Detroit.
The diagnosis of drug hypersensitivity has been handicapped by the inability of tests to detect the presence of antibodies. In the studies to be reported, salicylate conjugates have been prepared in the hope that these would prove to be effective immunogenic as well as reactive antigens. Antisera prepared in rabbits against the conjugates contained antibodies capable of reacting specifically with the salicylate portion of the conjugate. In addition guinea pigs were readily sensitized with salicylate conjugates and subjected to fatal anaphylactic shock by the intra-venous injection of salicylate conjugated to a heterologous carrier. The conjugated antigens were able to detect precipitating antibodies to low titer in the sera of two individuals with clinical allergy to aspirin. When tanned sheep red blood cells were treated with the conjugates, hemagglutinating antibodies were detected in the sera of five of six patients with aspirin sensitivity. Results of tests on normal sera suggest that the hemagglutination technique can be a reliable tool in the diagnosis of salicylate sensitivity.

11 *Immunology*

STUDIES ON A PRECIPITIN REACTION WITH HUMAN ANTIBODY TO RAGWEED.
Robert Feinberg and John Cline, Capt., MC (intr. by Sidney Gaines.) Walter Reed Army Institute of Research, Washington, D. C.
A ring precipitin test for blocking antibody to ragweed has been developed and applied to the sera of atopic individuals and of a non-atopic individual injected with extracts of short ragweed over a two year period. The test was carried out at very low ionic strengths by employing the pseudoglobulin fraction (Feinberg, R. & Silverstein, A., Fed.Proc.1959) of human serum and a ragweed extract which had been exhaustively dialyzed against distilled water. The post immunization serum from the non-atopic individual gave a positive precipitin reaction which was not given by the preimmunization serum. No precipitate could be demonstrated in any of these sera under isotonic conditions. The post immunization serum has blocking antibody in that it inhibits the passive transfer reaction of reaginic sera, inhibits the precipitation of rabbit anti-ragweed serum by its homologous antigen, and hemagglutinates with ragweed sensitized tanned red blood cells and bisdiazotized benzidine treated cells. This serum does not contain skin sensitizing antibody. The results of the tests on several paired sera from atopic individuals prior to and following hyposensitization will be presented and theoretical implications of the work discussed.

12 *Immunology*

IMMUNOELECTROPHORETIC STUDIES OF SERUM SICKNESS ANTIBODIES.
Noel R. Rose and Carl E. Arbesman*. Departments of Bacteriology and Immunology, and of Medicine, University of Buffalo School of Medicine, Buffalo, N. Y.
The sera of 20 patients injected prophylactically with tetanus antitoxin were tested for precipitating horse serum antibodies by immunoelectrophoresis as well as by tanned cell hemagglutination. Eleven patients had clinical symptoms of serum sickness. All of these individuals gave hemagglutination titers greater than 2000; only one of the other patients had a titer above this figure. Four patients' sera provided visible reactions in immunoelectrophoresis experiments. All 4 had developed serum sickness and had hemagglutination titers of 100,000 or greater. Two sera showed only one precipitation arc with a single horse serum component having the mobility of an α-2-globulin; one precipitated both in the α-2-globulin and γ-globulin zones and one precipitated only in the γ-globulin range. The same reactions appeared using 2 commercial antitoxins as antigens. Pre-injection samples available from 2 of the patients were negative, although hemagglutination titers of 200 or greater were found in both. The precipitating serum sickness antibody itself migrates in the globulin fraction. (Supported in part by research grant E 1303 (C2R1) from N.I.H.)

13 *Immunology*

CINEMICROGRAPHIC OBSERVATIONS OF IMMEDIATE-TYPE HYPERSENSITIVITY REACTIONS OF IRIS EPITHELIUM.
C. D. Dukes, H. H. Fox and L. J. Girard. (intr. by K. L. Burdon) Baylor Univ. College of Med., Houston, Texas. (Motion Picture)
It has been assumed that immediate-type hypersensitivity reactions are restricted to the so-called "shock-organs", smooth muscle and vascular endothelium. When time-lapse cinemicrography is employed, a specific allergic reaction can be demonstrated with other tissues. Iris epithelium from both normal and horse serum-sensitized guinea pigs were cultured in perfusion chambers for 72 hours. When these tissues were exposed to a 10 per cent solution of horse serum the following results were observed. (1) sensitized tissue cultured in sensitized serum; marked reaction: (2) sensitized tissue cultured in normal serum; questionable reaction: (3) normal tissue cultured in normal serum; no reaction: (4) normal tissue cultured in sensitized serum; moderate reaction. Sensitized tissue cultured in normal serum and exposed to 10 mcg per ml solution of histamine displayed a marked reaction, comparable to (1). These observations suggest that tissues other than the classic "shock-organs" are capable of exhibiting allergic reactivity and that this reactivity is dependent upon the presence of specific antibody. (Supported by a grant from the Council for Research on Glaucoma and Allied Diseases, Alfred P. Sloan Foundation, Inc.)

14 *Immunology*

THE PROGRESSIVE IMMUNIZATION REACTION IN THE RABBIT EYE.
Arthur M. Silverstein, Lorenz E. Zimmerman, and Sylvia Welter (intr. by F.D. Maurer) Armed Forces Inst. Path. Washington, D.C.
The injection of suitable amounts of protein antigens into the vitreous of the normal rabbit eye leads to the development of an inflammatory reaction in the uvea about 1 week later. The inflammation generally reaches a peak within 1-3 days, and slowly subsides over the following 7-10 days. Histopathologically, the cellular infiltrate is characterized from the start by mononuclear elements which undergo a marked plasma cell differentiation within 3-5 days following the onset of symptoms. Circulating antibody is usually not detectable in these animals until 1-2 days after initiation of ocular inflammation. With smaller doses of intravitreal antigen, clinical ocular response may be absent, or may appear only later and in a more subdued form after antibody has appeared in the circulation. Since antigen persists in the vitreous for relatively long periods, continuous antigenic stimulation occurs during the time that delayed hypersensitivity (?) and antibody production are developing. The ocular lesions appear to result from the process of local ocular antibody production, perhaps superimposed upon a hypersensitivity reaction. (Supported in part by a grant from the Council for Research in Glaucoma and Allied Diseases, Alfred P. Sloan Foundation, Inc.)

15 *Immunology*

IMMUNE RESPONSES TO REPOSITORY INJECTIONS OF RAGWEED ANTIGENS, H. L. Criep and H. Friedman*, V.A. Hosp. and Univ. of Pittsburgh, Sch. of Med., Pittsburgh, and Albert Einstein Med. Center, Phila. Pa.

Injections of ragweed pollen antigens as a water-in-oil emulsion into experimental rabbits and into allergic and non-allergic persons have resulted in immune responses markedly greater than that following injections of standard aqueous preparations as measured by various in vitro serological procedures. Immunization of rabbits with pollen extract in Freund's adjuvant resulted in a twenty fold or greater rise in hemagglutination titer, as well as increased complement fixing activity against ragweed antigens and precipitins as determined by tube precipitin and by gel diffusion tests. Injections of various groups of hay fever patients with a single repository injection of ragweed resulted in measurable hemagglutination titers using the bis-diazotized-benzidine method of coupling ragweed antigen to erythrocytes. Sera obtained prior to treatment and at various intervals following injection, prior to and following the season were assayed. A constant rise in titers was noted in the majority of patients receiving a repository injection during the first months following treatment. Persons who had been previously at the highest dosage level of aqueous injections gave similar responses as those who had never been injected with pollen extract. Titers rose to a range of 10^2-10^3.

16 *Immunology*

STUDIES ON THE SIGNIFICANCE OF FIBRINOLYSIS IN GUINEA PIG ANAPHYLAXIS. K. L. Burdon, J. P. McGovern,* G. D. Barkin,* and W. M. Meyers.* Dept. of Microbiology, Baylor U. Col. of Med., Houston, Texas.

The spontaneous lysis of clotted specimens of whole blood from guinea pigs during anaphylactic and anaphylactoid shock, and during related control experiments, was observed. Parallel samples were mixed with an equal amount of veronal buffer, pH 7.4, and with buffer containing protamine sulfate (200 μg/ml of blood mixture), and incubated for 24 hrs. at 37 C. A marked increase in clot lysis over the minimal amount in blood from normal animals occurred in all guinea pigs subjected to acute fatal anaphylactic shock. The thrombolysis was progressively greater in successive specimens collected during shock. Similar thrombolytic activity was observed in samples from normal animals during histamine, peptone, trypsin, and anaphylactoxin shock. Guinea pigs killed by suffocation also exhibited similar findings, thus indicating that the abnormal thrombolysis may be attributable, in part at least, to anoxia alone. Moreover, an excessive clot lysis was induced in normal guinea pigs by injection of human plasminogen activator without causing any signs of anaphylaxis. While activation of fibrinolysin in clotted blood samples is a regular feature of guinea pig anaphylactic and anaphylactoid shock, it does not appear to have a primary role in mechanism of these reactions.

17 *Immunology*

IS DELAYED HYPERSENSITIVITY AN INTERMEDIATE STAGE IN DEVELOPMENT OF CIRCULATING ANTIBODY? S. B. Salvin and R. F. Smith*. Rocky Mountain Laboratory, Hamilton, Mont.

Injection of 5 μg hapten-hen egg albumin conjugate (hap_1HEA) in Freund's adjuvant into the footpads of guinea pigs produces delayed hypersensitivity in 5 days and circulating antibody in 9 days after sensitization. Injection of 0.5-1.0 μg hap_1HEA in saline produces delayed hypersensitivity, but no detectable circulating antibody. In both cases, delayed hypersensitivity develops to the protein moiety but not to the hapten. The specificity of the circulating antibody, however, is directed to the hapten. When a primary dose of 0.5 μg hap_1HEA in saline is followed 8-10 days later by a secondary dose of 5.0 μg hap_1HEA in adjuvant, an anamnestic response results, with circulating antibody appearing on the fifth day after the secondary injection. Similarly, when a primary injection of HEA or of heterologous hapten-homologous protein (hap_2HEA), both of which develop delayed hypersensitivity to hap_1HEA, is followed by a secondary injection of hap_1HEA, an anamnestic response occurs. When, however, hap_1, attached to a heterologous protein such as bovine gamma globulin, is used as a primary dose to hap_1HEA, delayed hypersensitivity to hap_1HEA does not develop after the primary injection and an anamnestic response does not follow. Thus, delayed hypersensitivity seems indicated as a step in formation of circulating antibody.

18 *Immunology*

POLIOVIRUS INHIBITION BY MONKEY NERVOUS TISSUE SUSPENSION. Samuel Baron and Richard J. Low (intr. by George A. Hottle.) Division of Biologics Standards, National Institutes of Health Bethesda, Md.

An unexpected low rate of virus isolation was observed during studies of rhesus monkey central nervous system (CNS) infected with attenuated polioviruses. When infected CNS (brain or spinal cord) was plaque-assayed on monkey kidney cell cultures more plaques appeared at 10^{-2} and 10^{-3} dilutions than at 10^{-1} dilution, indicating virus inhibition. Similarly normal CNS suspension inhibited 10 to 100 added PFU of each of the 3 types of poliovirus. In addition poliovirus plaque size was significantly decreased when 1 to 75 final dilution of CNS suspension containing inhibitor was present in the overlay medium. No inactivation of poliovirus occurred when inhibitor and virus were incubated together at 37°C for 2 hours and then diluted beyond inhibition before virus assay. This result suggests that inhibitor does not irreversably inactivate poliovirus and that the inhibitor is not antibody. Coxsackie A 9 and Echo 12 viruses were not inhibited by the CNS suspension. The presence of CNS inhibitor may explain some reported failures of poliovirus recovery from the CNS monkeys and may relate to the pathogenesis of poliovirus infections of the CNS.

19 *Immunology*

A STUDY OF IMMUNOCHEMICAL SPECIFICITY IN DELAYED HYPERSENSITIVITY. Sidney Leskowitz. Harvard Med. School, Boston, Mass.

Guinea pigs and rabbits, sensitized with bovine serum albumin (BSA) in complete Freund adjuvant, show both Arthus and delayed reactions on subsequent skin test with BSA. When, however, the skin test was done with antigen in the form of a specific precipitate prepared with excess rabbit, guinea pig or mouse antibody, the Arthus reaction was completely suppressed, and only the characteristic, though more sharply defined, delayed reaction was seen. A comparison of the effect of other alterations in the structure of BSA on its ability to react with antibody and to elicit delayed hypersensitivity was made. Rupture of disulfide bonds by heat or thioethanol markedly affected the antibody but not the delayed-type reactivity. Addition of new chemical groupings by iodination or dinitrophenylation produced similar effects. The results indicate that the ability to elicit delayed hypersensitivity is apparently less affected by structural changes in the protein than is antibody reactivity. The theoretical implications of these findings will be discussed.

(Supported by a grant from the NIH)

20 *Immunology*

ANTIBODY PRODUCTION AND SKIN HYPERSENSITIVITY FOLLOWING VACCINIA INFECTION IN GUINEA PIGS. Robert Friedman and Samuel Baron (intr. by C. P. Li.) Division of Biologics Standards, National Institutes of Health, Bethesda, Md.

Interest in the relationship between delayed skin hypersensitivity and antibody production in viral systems prompted investigation into the development of these phenomena in guinea pigs during infection with vaccinia virus. Reliable determination of early antibody production seemed possible employing markedly sensitive viral plaque neutralization methods. Guinea pigs infected with chick embryo grown vaccinia were bled and skin tested with monkey kidney (MK)tissue culture grown, heat inactivated virus. Serum was tested for antibody by plaque neutralization in MK bottles. Following intradermal (ID) inoculation of 10^7 plaque forming units of vaccinia virus skin infection occurred and was maximal on the 6th day. Delayed skin hypersensitivity and neutralizing antibody were not detected until the 4th day and immediate skin hypersensitivity was not observed during the 18 days of the experiment. In contrast to studies employing non-living protein antigens, this study shows early appearance of both antibody and delayed hypersensitivity to viral antigens.

XUM

21 *Immunology*

CELLULAR TRANSFER BETWEEN HOMOZYGOUS AND HETEROZYGOUS GUINEA PIGS WITH REGARD TO CONTACT-TYPE HYPERSENSITIVITY AND ANTIBODY PRODUCTION. Merrill W. Chase. Rockefeller Institute, New York.

With the idea that transfer of living cells of the lymph nodes and spleens between guinea pigs should incur homograft rejection unless donor and recipient were homozygous, transfers were made within and between the highly inbred Wright strains II and XIII, and between XIII and the Hartley strain. Donor groups were subjected to the "Combination method" of sensitization to picryl chloride (Intern.Arch.Allergy 5,163, 1954), allowing tests on recipients for contact-type hypersensitivity with picryl chloride and tuberculin hypersensitivity. Contact tests were made on days 1, 2, 6 and 20 following transfer, tuberculin tests (50 mcg PPD-S) on days 2 and 20, and small bleedings were taken on days 6, 11 and 22 to allow assessment of anti-picryl antibody by means of PCA using as antigen picrylated casein. Homozygous XIII transfers produced high, enduring contact reactivity and Ab to picryl increased, also tuberculin reactivity was strong and increased, unlike results with heterozygous stocks used before. II/II transfers were much the same but with less tuberculin sensitivity. With XIII/II and XIII/Hartley transfers, early-phase contact reactions waned and lesser Ab appeared. Contrary to the basic assumption, II/XIII transfers produced excellent, ascending tuberculin reactivity;Ab was low despite good contact tests.

141. IMMUNE RESPONSE—Hypersensitivity—Anaphylaxis

1 *Physiology*

HISTAMINE RELEASE AND INTRACELLULAR POTENTIALS DURING ANAPHYLAXIS IN THE ISOLATED HEART. George A. Feigen, E. M. Vaughan Williams*, Janice K. Peterson*, and C. B. Nielsen. Physiology Department, Stanford University.

The response of the sensitized guinea pig heart to perfusion with an effective dose of antigen was found to consist of an acceleration of the rate, an increase in the amplitude of contraction, and a decrease in coronary flow. The characteristic mechanical reaction of the isolated atria was an increase in amplitude and frequency of contraction, the more intense effects resulting in fibrillation; electrophysiologically, atrial hyperexcitability was demonstrable by bursts of action potentials, recurring as soon as the membrane had repolarized sufficiently to be re-excited. The resting potential was reduced and the overshoot and the rate of rise of the action potential were diminished, suggesting that the membrane had become very permeable to sodium during the acute phase of shock. All of the mechanical and electrical events noted in the Langendorff heart and the atrial preparations during anaphylaxis could be reproduced precisely by an appropriate dose of histamine. Evidence for the release of histamine by both the perfused heart and the isolated atria was first obtained by pharmacological methods and then confirmed by paper chromatography of butanolic extracts.

2 *Physiology*

COMPARATIVE EFFECTS OF FORWARD ACCELERATION AND EXERCISE. A. R. Slonim (intr. by R.F. Kline). Acceleration Section, Biophysics Branch, Aerospace Med. Lab., Wright Air Development Division, Wright-Patterson AFB, Ohio.

Five fasting subjects were exposed to forward accelerations on the human centrifuge (head and trunk inclined 12° in direction of acceleration) higher than those previously reported (Slonim, J.A.P. 15:No.1, 1960) to observe changes in body fluid levels of constituents which might reflect increased muscle activity. These subjects were also exercised for 10 mins. on a treadmill at an 8% grade and a speed of 3.5 miles per hour. The blood levels of creatinine, glucose, inorganic phosphate and plasma bicarbonate were not significantly altered under centrifugation. The blood creatinine was significantly increased and plasma bicarbonate decreased with exercise. Blood glucose and phosphorus levels were not consistently altered. There were no abnormalities in the urine, no significant changes in urine creatinine nor consistent changes in urine volume in the pre-control, test and post-control periods of both groups. By these measures severe forward acceleration is not comparable to heavy exercise. These accelerations caused marked subjective fatigue which does not correlate with the biochemical indices measured in these studies.

3 *Pharmacology*

PHARMACODYNAMICS OF N-P-CHLORO-BENZHYDRYL-N'-METHYL HOMOPIPERAZINE DIHYDROCHLORIDE (HOMOCHLORCYCLIZINE;SA-97). Eugene T. Kimura, Patrick R. Young*, and Richard K. Richards. Dept. of Pharmacology, Abbott Labs., No. Chicago, Ill.

Studies on this homologue of chlorcyclizine showed it to have antagonistic action towards (1) Brocklehurst's slow-reacting-substance (SRS-A), (2) serotonin, (3) acetylcholine and (4) histamine. The anti-serotonin potency of homochlorcyclizine was similar to BAS (benzyl analog of serotonin), both in vitro and in vivo. A concentration of 4 X 10^{-6} gm./ml. of the drug reduced the spasmogenic activity of SRS-A. It shielded guinea pigs against both passive and active anaphylaxis and showed coronary dilator and antifibrillatory properties. A biphasic depressant-stimulant action was noted on the CNS of mice, rats and dogs both acutely and chronically. Its CNS stimulant properties in mice were in marked contrast to the depression shown by BAS. Barbiturate sleep times were initially prolonged and secondarily decreased by prior treatment with the drug. Homochlorcyclizine showed weak or no activity in the following tests: antipyresis, analgesia, anti-emesis and MO-inhibition. Acute toxicities were determined in mice, rats, guinea pigs, rabbits, cats and dogs; chronic toxicities were run in rats and dogs.

4 *Pharmacology*

HISTAMINE AND 'SLOW REACTING SUBSTANCE' IN ANAPHYLACTIC REACTION. N. Chakravarty and B. Uvnäs (Intr. by L.S. Goodman.) Depts. of Pharmacol., Univ. of Ottawa, Canada, and Karolinska Institutet, Stockholm, Sweden.

On incubating sensitized guinea pig tissue with antigen, not only histamine, but also another smooth muscle stimulating principle ('slow reacting substance' or 'SRS') diffuses into the surrounding fluid. This report is based on studies on the effect of changes in the external milieu on the appearance of histamine and 'SRS' in anaphylactic reaction in vitro. The appearance of both histamine and 'SRS' was pH and temperature dependent - the optimal pH being between 7 and 8.5 and optimal temperature about 37°C. Oxygen and calcium were necessary for both. When histamine release was inhibited by various substances - e.g. polymeric compounds, ethanol, organic dyes or well known enzyme inhibitors - there was a concomitant inhibition of the occurrence of 'SRS'. From the nature of the enzyme inhibitors used it seems that sulfhydryl and amino groups of proteins are involved in the reaction. The observations are compatible with the hypothesis that the activation of a common enzymatic pathway in the mast cells by antigen-antibody reaction leads to the appearance of both histamine and 'SRS'.

5 *Immunology*

ANTIBODY FIXATION. Zoltan Ovary. Department of Pathology, New York University.

Rabbit antibody mixed with its specific antigen (in excess) cannot give PCA reactions. However, this "neutralized antibody" can be fixed to guinea pig tissues since reverse PCA (RPCA) reactions can be provoked with anti-rabbit gamma globulin. When rabbit antibody is mixed with excess of horse anti-rabbit gamma globulin neither PCA nor RPCA reactions can be obtained. This suggests that horse anti-rabbit gamma globulin covers the antibody combining sites and/or the "fixation site". Papain digestion has been shown to split rabbit antibody into 3 fragments, two of which are very similar in having one antibody combining site and some antigenic determinants characteristic for rabbit gamma globulin; the third one has no antibody combining site but most of the antigenic determinants. The two (similar) fragments containing the antibody combining site do not give PCA or RPCA reactions, while the third one gives good RPCA reactions with anti-rabbit gamma globulin. The "fixation site" is therefore on the third fragment.

6 *Immunology*

PASSIVE HOMOLOGOUS ANAPHYLAXIS IN THE MOUSE: STUDIES ON THE "LATENT PERIOD". P.E. Treadwell* (intr. by A.F.Rasmussen, Jr.) Division of Virology, Dept. of Infect. Dis., School of Med., Univ. Calif., Los Angeles.

Mouse anaphylaxis can be produced (1) by passive administration of antibody followed by antigen; (2) by active sensitization followed by antigen; (3) or by intravenous injection of antigen-antibody complexes, In order to compare anaphylaxis produced by injection of antigen into passively sensitized mice with anaphylaxis produced by other methods, four different doses of homologous antibody to Bovine Plasma Albumin (BPA) were injected intravenously. The sensitized mice were then shocked intravenously, immediately and 2,6,24,48,72, and 168 hours later with 1,10, or 100 times the equivalence dose of BPA. The results indicate that (1) passive anaphylaxis is dependent upon the amount of antibody, the amount of antigen, and the time interval between injections; (2) a "latent period" of variable onset and duration results from too little or too much of either antigen or antibody; (3) passive anaphylaxis in the mouse is primarily due to the formation of circulating antigen-antibody complexes in the first 24-48 hours after sensitization and to a tissue antibody-antigen reaction after this period. (Supported by grants from National Institutes of Health.)

7 *Immunology*

STUDIES ON THE MECHANISM OF ANAPHYLAXIS IN THE MOUSE. S. Tokuda*■ and R. S. Weiser, Dept. of Microbiology, Univ. of Washington, Seattle.

Evidence will be presented to support the concept that 5-hydroxytryptamine (5HT) plays an important role in anaphylaxis in mice induced by i.v. injection of soluble complexes of BSA and anti-BSA rabbit antibody. Mice treated with reserpine 18 to 22 hours before challenge showed little or no evidence of shock. This protection apparently resulted from 5HT depletion because the refractoriness of "reserpinized" animals was reversed by treatment with 5-HTP but not with DOPA. Pretreatment with the anti-5HT agents, LSD, BOL and Chlorpromazine, also gave protection against shock. Trials with other agents will be presented, including trials with certain antihistaminics which proved to be ineffective. Attempts to protect pertussis-vaccinated mice by pretreatment with reserpine and BOL were unsuccessful. Mice given a preliminary sublethal dose of the complex were highly refractory to subsequent challenge with the complex. Attempts to overcome this refractoriness with 5-HTP and complement and a combination of both agents failed. No morphological alterations in mast cells were detected following anaphylaxis.

■Graduate Trainee; Research Training Grant CRT5040, Nat. Can. Inst. U.S.P.H.S.

8 *Immunology*

PASSIVE CUTANEOUS ANAPHYLAXIS IN GUINEA PIGS SENSITIZED WITH SOLUTIONS OF ANTIGEN AND ANTIBODY. Leon T. Rosenberg* and Sidney Raffel. Stanford Univ. School of Med., Stanford, Calif.

Solutions of guinea pig antibody and excess antigen (egg albumin) have been reported to sensitize guinea pig skin (Proc. Soc. Biol. Med. 98:451,1958). Subsequent injection of antigen elicits PCA. In the present study, bovine serum albumin in excess and guinea pig antibody were injected intracutaneously to sensitize animals. These were challenged with antigen and dye, or dye alone, at hourly intervals. The time required for sensitization by complex solutions is greater than the latent period for sensitization with free antibody. In addition, animals challenged before sensitivity was well established showed a delay in dye accumulation at sensitized sites. Dye localization occurred over a period of an hour or more instead of the 2-15 minutes characteristic of sites sensitized with antibody alone.

9 *Immunology*

HISTAMINE, 5-HYDROXYTRYPTAMINE AND CORTISOL IN MOUSE ANAPHYLAXIS. R. D. Higginbotham (intr. by A. Packchanian). Dept. Microbiology, Univ. Texas Medical Branch, Galveston, Texas.

Histamine (H) and 5-hydroxytryptamine (5HT) are present in mast cells of the mouse and may be released from this source during anaphylaxis. One or both amines may be impt. mediators of anaphylactic shock in this specie. Adrenalectomy will markedly reduce resistance of mice to anaphylaxis and this effect can be inhibited by cortisol. It was found that adrex decreased resistance to 5HT more markedly than to H (6.5x vs 1.2x) and 5HT was approx. 10x more lethal to adrex mice than H (LD_{50}'s: 20 vs 275 µg/gm). Cortisol protected mice more effectively against 5HT than H. On the other hand, assoc. studies indicated that adrenalectomy decreased resistance to anaphylaxis more markedly than to 5HT (100x vs 6.5x) and that anaphylaxis was more resistant to cortisol therapy than was 5HT intoxication. (Supported by grants from Medical Research Foundation of Texas and Burroughs Wellcome & Co., Inc.).

10 *Immunology*

ENDOTOXIN AND ITS RELATIONSHIP TO ANAPHYLAXIS IN THE MOUSE. Julia M. Finbinder*, Charles L. Fox, Jr., and Carl T. Nelson, Dept. of Dermatology, Columbia Univ., N.Y., and, Dept. of Surgery, New York Med. Coll.-Flower & Fifth Ave. Hosps., N.Y.

There are reports of analogous findings in endotoxin shock and in trauma, burn and hemorrhagic shock. However, the relationship of endotoxin to anaphylaxis (ANA) has not been as intensively studied. This study indicates that a non-lethal dose of endotoxin (E. coli - DIFCO 5-25 µ) given to hypersensitive mice 1½ to 3 hours pre-challenge, markedly decreases the amount of challenging antigen needed to produce fatal ANA. Endotoxin also decreases the interval between completion of sensitization and susceptibility to fatal challenge as well as the number of sensitizing injections required to achieve high susceptibility to ANA. Drugs which effectively protect mice against fatal ANA are protective against endotoxin. A more specific relation of endotoxin to ANA may be inferred from the demonstration that hypersensitive mice, tolerant to endotoxin, will not succumb to ANA when challenged. Furthermore, a small dose of endotoxin (25 µ or less) given 24 hours pre-challenge, protects mice from the lethal results of antigen-antibody union. This is the period when mice would be refractory to a second dose of endotoxin. In the latter two cases antigen-antibody union has occurred. It appears as if antigen-antibody union creates a chain of events similar to an endotoxin injection.

11 *Immunology*

GENETIC INFLUENCE ON CIRCULATING ANTIBODY AND ANAPHYLAXIS IN THE MOUSE. Richard M. Rothberg* and David W. Talmage. Dept. of Medicine, Univ. of Chicago.

Using 1.0 mg. bovine serum albumin (BSA) in oil adjuvant to sensitize mice and measuring the antibody response as antigen binding capacity (ABC) with the ammonium sulfate salting out technique (J. Inf. Dis., 1958, 103:239), experiments were conducted to provide additional information regarding the relationship of circulating antibody to anaphylaxis and the influence of genetic background on both anaphylaxis and production of circulating antibody. Evidence will be presented to demonstrate that: (a) C_{57} and DBA mice produced only small amounts of circulating anti-BSA (ABC-33 @ 0.2 ug = 1.6 to 3.3 ug BSA N/ml.), and anaphylaxis was rarely demonstrated with these strains. (b) CF_1 mice produced more antibody (ABC-33 @ 0.2 ug = 27.5 ug BSA N/ml.) and were highly sensitive to anaphylaxis. (c) A 2.0 mg. immunizing dose of BSA resulted in an earlier antibody response and earlier anaphylaxis with CF_1 mice than did the 1.0 mg. immunizing dose. (d) The severity of anaphylaxis observed with all 3 strains appeared to be related to the amount of antibody present. (e) Anaphylactic shock was not observed in any strain until after circulating antibody could be demonstrated.

XUM

142. IMMUNE RESPONSE– Tissue Transplants

1 *Physiology*

AUTOGRAFTS AND HOMOGRAFTS OF THE STOMACH IN DOGS. R.C. Lillehei, J.K. Longerbeam, and B. Goott. (intr. by J. Johnson.) Dept. of Surg., Univ. Minnesota Med. School.

Previously we described techniques of preparing autografts and homografts of the small bowel in dogs. The bowel autografts functioned indefinitely although no effort was made to re-establish lymphatic or nervous connections to the autografts. Bowel homografts also functioned for periods up to 8 days. Similar techniques have been used to construct autografts and homografts of the entire stomach. Gastric autografts secrete acid and pepsin promptly following their construction and dogs having such grafts have so far survived up to 9 months. Gastric homografts also secrete acid and pepsin following construction but only until rejection occurs in 8 days. Studies are presently in progress to evaluate the effect of prolonged in vitro hypothermia on the function and survival of such grafts. (Supported by a grant from the U.S.P.H.S.)

2 *Biochemistry*

THE INFLUENCE OF 6-MERCAPTOPURINE ON SURVIVAL OF HOMOLOGOUS RABBIT KIDNEY TRANSPLANTS. P. Nathan*, E. Gonzalez*, H. Pescovitz*, R. Fowler, Jr.*, and Benj. F. Miller, May Institute for Medical Research of the Jewish Hosp. of Cincinnati; and the Depts. of Physiol. and Med., Univ. of Cincinnati Coll. of Med.

Kidneys from Dutch rabbits were transplanted by vascular anastomoses to New Zealand albino rabbits. Five recipients were treated with 6-mercaptopurine (6MP), 12 mgm/kgm daily for 7 to 14 days, to test this drug's ability to produce immunologic tolerance to organ transplants. Schwartz and Dameshek reported that 6MP induces tolerance to soluble protein antigens. Seven homotransplants were prepared in untreated hosts. The transplants in the experimental and control animals showed lymphoid cell accumulation and tissue necrosis. Despite the cellular destruction, all of the 6MP treated rabbits and 2 of the 7 controls retained a blood flow through the kidney medulla for 18 to 38 days when the individual experiments were terminated. Presumably the renal blood vessels in the rabbit remain patent because their medullary vasculature can shunt a large part of the kidney blood supply away from the destroyed cortex. Although the 6MP treated hosts destroyed a major portion of the transplant, a number of renal parenchymal cells survived over 24 days despite the specific immune reaction occurring within the transplant. (Supported in part by the Amer. Heart Assoc.; the Josiah Macy, Jr. Fdn., and U.S.P.H.S. Grant No. A-2938)

3 *Pharmacology*

DELAYED INTRACUTANEOUS HYPERSENSITIVITY TO DONOR LEUKOCYTES FOLLOWING REJECTION OF SKIN HOMOGRAFTS IN MAN. John P. Merrill, Eli A. Friedman, Richard Wilson, and Colette Hanau. (intr. by D.G. Friend.) Peter Bent Brig. Hos. and Harvard Med. School, Boston, Mass.

Eleven human volunteers rejected full thickness homografts in 4 - 26 days. After rejection each of the patients was intradermally tested with autologous, donor, and nonspecific homologous red cells, white cells and plasma obtained from peripheral blood. A reaction was considered positive if at least 10 mm of induration was detected by two observers. Positive reactions appeared at 12 hours, were maximal at 24 - 48 hours. All subjects had positive reactions to donor leukocytes. One reacted also to autologous leukocytes. Nine had greater reactions to nonrelated cells than to specific donor leukocytes. Fifty-four "control" patients including 10 normal subjects and ten recent recipients of multiple blood transfusions all had negative reactions. None of the grafted or "control" group reacted to red cells or to plasma. This suggests that a delayed hypersensitivity manifested by dermal reaction to leukocytes may be induced by homograft rejection in the human, as previously shown for the guinea pig. Though the magnitude of the reaction is greatest with specific donor cells the reaction is not entirely individual specific.

4 *Pharmacology*

BEHAVIOR OF L-FIBROBLASTS AND SARCOMA 180 CELLS IN DIFFUSION CHAMBERS IN RESISTANT HOSTS. John D. Gabourel (intr. by A. Goldstein.) Dept. of Pharmacology, Stanford, Calif.

L-fibroblasts and sarcoma 180 cells from in vitro culture have been grown (log phase) in diffusion chambers implanted i.p. in Swiss-Webster mice (in press, Cancer Research) and in C3H mice. L-fibroblasts do not produce tumors in either strain in contrast to S-180 cells which produce a high incidence of tumor in both strains (i.p. or s.c.). A homograft type reaction to the L-fibroblast has been suggested to explain these findings. Studies made with hosts pre-treated with L-fibroblasts show the following: no depression of growth while rejection presumably analogous to either first-set or second-set reactions are occurring outside the chamber. However, if the mice were "hyper-immunized" with L-fibroblasts, growth of these cells in the diffusion chamber was markedly depressed. Such pretreatment with L-fibroblasts only slightly depresses the growth of S-180 cells in diffusion chambers. In mice "hyper-immunized" with S-180 cells (subcutaneous inoculation of 1 x 10^7 cells did not produce tumors in these animals) the growth of L-fibroblasts in diffusion chambers was markedly depressed whereas the growth of S-180 cells in this system was equivalent to that in chambers placed in untreated hosts. (Supported by Grant T-38 Amer. Cancer Soc.)

5 *Pathology*

LYMPHATIC TISSUE REACTION IN NORMAL MICE GIVEN FOREIGN BONE MARROW. C. C. Congdon. Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee.

Loss or dissociation of germinal centers in the spleen white pulp is the first change seen in normal mice given a large intravenous injection of foreign bone marrow. The swollen white pulp is then replaced by antibody-forming cells. Nearly all lymphocytes disappear. The antibody-forming cells then disappear and regeneration of the germinal center occurs. Lymphocytes again fill the white pulp. Collections of plasma cells in or near the white pulp can be seen. Rate of delivery of antigenic material possibly controls the amount of plasma cell formation as it does the hyperplasia of germinal centers during their regeneration. After rat bone marrow injection most of the antibody-forming cells have disappeared in 4 days; with the same amount of homologous bone marrow, these cells take 9 or 10 days to disappear. Normal mice given rat serum, bovine serum albumin, sheep red blood cell ghosts, Salmonella endotoxin, bacterial polysaccharide, and pertussis vaccine show essentially the same alterations in the splenic white pulp.

6 *Pathology*

ROLE OF MYCOPLASMA (PPLO) IN THE REGRESSION OF MURPHY-STURM LYMPHOSARCOMA IN THE RAT. E. Virgil Howell* and Russell S. Jones. Dept. of Path., Univ. Utah Coll. Med., Salt Lake City, Utah.

Mycoplasma or pleuropneumonia-like organisms (PPLO) have been isolated from Murphy-Sturm lymphosarcoma in rats (Holtzman) as well as from the polyarthritic lesions associated with the spontaneous or induced regression of this tumor (Proc. Soc. 102:210, 1959). Experiments were designed to evaluate the interrelationships of the PPLO in the tumor and in the host, focal necrosis in the tumor, tumor regression and tumor resistance. After tetracycline therapy the lymphosarcoma was culturally free of PPLO. Both "PPLO-free" and "PPLO-infected" tumors were carried by transplantation. Rats bearing "PPLO-free" tumors were infected with PPLO at various intervals before and after tumor transplantation. The PPLO-free tumors were resistant to infection by PPLO unless partially necrotic or of large size. The presence of PPLO in the tumor did not lead to necrosis and only mildly increased the incidence of spontaneous regression. However, the presence of PPLO led to enhanced susceptibility of the tumor to the necrotizing action of K. pneumoniae polysaccharide and other substances.

7 *Pathology*

PARABIOSIS INDUCED TOLERANCE. Ronald D. Guttmann and J. Bradley Aust (intr. by J. Dawson). U. of Minn. Med. School

Homologous tumors injected into newborn mice of inbred strains will take, grow, and metastasize and eventually destroy their hosts. In an endeavor to cause regression of these tumors when their hosts reached adulthood, they were put in parabiosis with non-tumor bearing isologous mice. After no regression was noted the non-tumor bearing partners were implanted with homologous tumor and found to be tolerant to the neoplasm. In a further attempt to induce tolerance in adult immunologically mature mice, parabiosis was made between animals of various strains and their F_1 hybrids. The F_1 hybrid accepts tissue from either parent strain but the inbred animal does not accept F_1 hybrid tissue. It was expected that the F_1 hybrid animal would tolerate parabiotic union with a parent strain animal but the reverse should not be true. This presumption was confirmed. All parabiotic deaths (after 15 days) were of the F_1 hybrid. However, not all F_1 hybrid animals died. Depending on the strain combinations employed, 0-60% of the F_1 hybrid: parent pairs lived in harmony. When the parent strain partners were tested with out-cross skin, they tolerated these grafts permanently. (Z-ZCeF_1 parabiosis; Z became tolerant to Ce skin). (Supported by a grant from USPHS).

8 *Pathology*

TIME LAPSE CINEMATOGRAPHY OF LYMPHOAGGLUTINATION AND LYMPHOLYSIS PRODUCED BY SERA FROM HOMOGRAFTED ANIMALS. Paul I. Terasaki, Jack A. Cannon, and William P. Longmire, Jr. (Intro. by Roy L. Walford) Univ. of Calif., Medical Center, Los Angeles, California.

Humoral antibodies directed against homografts have generally been difficult to demonstrate. With the use of dark-field and phase-contrast time-lapse cinematography, it has been possible to demonstrate agglutination and death of homologous lymphocytes produced by sera from animals injected with pooled homologous (chicken) splenic tissue. Since lymphocytes are normally very actively motile cells, a strong force can be seen to be exerted by the cells in "attempting" to pull apart. Death of lymphocytes can accurately be determined by continuous observations of the motility of the cells. The cytotoxic effect of sera from grafted animals has been assayed using motility as the criterion of viability, since other gross histological changes are probably post mortem changes which may take some time to develop after the actual death of a cell. These movies establish that sera from homografted animals very often react upon lymphocytes of the donor animal causing agglutination and death of cells. (Supported by a grant from U.S.P.H.S. #A-2375)

9 *Pathology*

MODIFICATION OF THE HOMOGRAFT RESPONSE IN MICE BY ASCITES. Robert E. McCarthy (intr. by G. E. Foley). Labs. of Microbiology, Children's Cancer Res. Fndn., and Dept. Pathology, Harvard Medical School, Boston, Mass.

Antigenic distinction between certain normal and neoplastic mouse cells derived either from fresh tissue or cell cultures is evidenced by differences in their capacity to sensitize homologous mice, as determined by responses to secondary skin homografts (McCarthy, et al., Fed. Proc., 18:611, 1959). During these studies it became apparent that factors independent of differences in antigenic structure influenced survival of the secondary graft. The reaction of the homologous host, as indicated by skin graft rejection time, appears to be delayed or inhibited in the presence of ascites. This modified response to skin grafts can be effected by ascites produced by cells which are either isologous or homologous with the primary sensitizing antigen. No such modification of the homograft reaction was effected by increased intraperitoneal fluid volume when produced by daily administration of saline solution. Consideration of the relationships between the site of the primary homograft, the presence or absence of ascites and their effect upon survival of the second homograft, indicates that development of physiological ascites in response to appropriate stimulation may block the immunological response of the host against certain homografts.

10 *Pathology*

PERSISTENCE OF CLASSICAL HOMOLOGOUS TISSUE REACTIONS DURING PSEUDOPREGNANCY. V. Truchly,* D. Madden,* S. Papadopoulos* and R. Andresen. Dept. Path. and Lab. Surg. Res., Presbyterian-St. Luke's Hospital, Chicago, Illinois.

Previous studies in rabbits showed that during pregnancy the customary inflammatory and destructive effects of the homograft reaction were eliminated so that sequences of degeneration, absorption and organization within the homograft of the pregnant host proceeded in a manner identical to tissue reactions of the animal to its own grafted tissues. In the present experiments a state of pseudopregnancy was initiated by two methods: first by injections of pregnant human urine and secondly by sterile matings. Musculofascial grafts were resected from male and non-pregnant females and implanted within 4 to 7 days following stimulation of the recipients. Two weeks later microscopic study of all homografts revealed typical incompatible tissue reactions. Corpora lutea were present in the ovaries and the uteri showed hyperplastic, hyperemic endometria. These studies have shown that the state of pseudopregnancy with its associated alteration of the upper genital tract is incapable of reproducing the harmonious modification of homologous tissue reactions so characteristic of pregnancy.

11 *Pathology*

ABSENCE OF CLASSICAL HOMOLOGOUS TISSUE REACTIONS FOLLOWING TRANSFUSIONS OF BLOOD FROM MULTIPLE DONORS OTHER THAN DONORS OF GRAFTS. R.H.Andresen, C.W.Monroe,* G.M.Hass, D. Madden* and S. Papadopoulos.* Dept. of Path. and Lab. of Surg. Research, Presbyterian-St. Luke's Hospital, Chicago, Illinois

Previous studies in rabbits showed that cross-transfusion prior to cross-grafting elicited a reproducible modification of classical homologous tissue reactions regardless of blood types. This consisted of fibrocollagenous encapsulation of the homograft with varying degrees of vascular penetration by the host in the absence of customary inflammation. In the present experiments the recipient was injected with 5 ml of blood from each of six random donors and then grafted with musculofascial tissues resected from non-blood donors. Two weeks later, microscopic study of the homografts showed typical post-transfusion tissue reactions 80% of the time. The remainder consisted of minimal focal necrotizing reactions superimposed upon the usual architecture of the post-transfusion graft. These studies have shown that it is possible by means of "in vivo pooling of blood" within a recipient prior to grafting to elicit a consistent modification of the classical response to homografts from all individuals of the same strain of rabbits.

12 *Pathology*

THE EFFECT OF 6-MERCAPTOPURINE ON THE HOMOGRAFT RESPONSE IN RABBITS. W.R. Meeker and R.L. Varco (intr. by R.A. Good) Univ. of Minnesota Medical School, Minneapolis, Minnesota.

6-mercaptopurine (6-MP) suppresses the primary immune response to bovine serum albumin in doses of 3 mg./kg./day or higher given to rabbits during antigenic stimulation, but has no effect on the secondary response. (Schwartz et al, J. Clin. Invest. 1959, 38:1394). In these studies the effect of 6-MP on the survival time of skin homografts exchanged between pairs of New Zealand and Dutch strain rabbits was noted. Dose levels of 3, 6, 12, and 25 mg./kg./day were used. Three treatment schedules were used: 1) Treatment for 2 weeks before grafting. 2) Treatment from the day of grafting to slough. 3)Treatment begun 5-7 days post-grafting to slough. 6-MP, 18 mg./kg./day, from the day of second set homografting to slough was also studied. Statistically significant prolongation of homograft survival occurred only in animals receiving 6 mg./kg./day or higher from the day of first set grafting and was maximal at 12 mg./kg./day. Dose levels and treatment schedules used are important in assessing the 6-MP effect on the homograft response which appears to be suppression during the induction of homograft immunity. (Supported by grants from U.S.P.H.S., Minnesota Heart Association, and American Heart Association)

XUM

13 *Immunology*

AN IMPROVED TECHNIQUE OF SKIN GRAFTING AND ITS FAVORABLE INFLUENCE ON GRAFT SURVIVAL. MORTON PADNOS, LEO GROSS, AND BERNARD GOTTFRIED (INTR. BY NORMAN MOLOMUT). WALDEMAR MEDICAL RESEARCH FOUNDATION, PORT WASHINGTON, NEW YORK.

A RELIABLE SURGICAL PROCEDURE FOR SKIN GRAFTING IN MICE HAS BEEN DEVISED. SCRUPULOUS SURGICAL TECHNIQUE RESULTS IN 100% SURVIVAL OF AUTO AND ISOGRAFTS. UTILIZING THIS METHOD WE HAVE RE-EXAMINED SEVERAL FACTORS REPORTED TO BE OPERATIVE IN SKIN GRAFT SURVIVAL, I.E., STATE OF SKIN CYCLE OF GRAFT AND RECIPIENT, THE FACTOR OF GRAFT THICKNESS, AND SEX DIFFERENCES. OUR RESULTS INDICATE THAT THESE FACTORS ARE NOT CRITICAL IN GRAFT SURVIVAL. FIVE INBRED STRAINS OF MICE, INVOLVING OVER 500 INDIVIDUAL MICE, WERE EMPLOYED FOR EXCHANGE SKIN GRAFTS BETWEEN INDIVIDUAL MICE OF THE SAME STRAIN. CONTROL GRAFTS WERE ISOGRAFTS. OUR RESULTS IN SEVERAL HUNDRED GRAFT SETS INDICATE THAT: SKIN GRAFTS OF DIFFERING STAGES OF SKIN CYCLE, AND FULL THICKNESS SKIN GRAFTS SURVIVE IN ALL CASES. MALE TO FEMALE SKIN GRAFTS SURVIVE IN A HIGHER PERCENTAGE THAN REPORTED. WE FIND THAT THE CRITICAL FACTORS ARE AN EXACT FIT OF THE GRAFT AND GRAFT BED, MAINTENANCE OF NORMAL SKIN TENSION, AND PREVENTION OF DISTURBANCE OF THE GRAFT BY THE ANIMAL'S MOVEMENTS AND/OR ATTEMPTS TO DISTURB THE GRAFT. THIS PROCEDURE PROVIDES A RELIABLE SKIN GRAFT METHOD FOR THE FURTHER STUDY OF IMMUNE, GENETIC AND PHYSIOLOGICAL FACTORS IN GRAFT SURVIVAL.

14 *Immunology*

STUDIES ON IMMUNOLOGIC ENHANCEMENT OF SARCOMA I.
R. S. Weiser and K. Kim* ■ Dept. of Microbiology, Univ. of Washington, Seattle.

The observations of Kalliss on enhancement of SaI in the normally resistant C57BL/6 mouse have been confirmed. In addition it was found that, although mice bearing enhanced tumors were immune to challenge with stock tumor, they failed to reject biopsied materials from their own tumors transplanted at distant sites. Whereas live spleen and lymph node cells from mice of the strain of tumor origin, Strong A(A/jax), administered at either the site of tumor implant or at a distant site suppressed the enhanced growth of tumor in "enhanced" mice, neither frozen and thawed cells or liver cells produced this effect. Enhancement was achieved with both the solid and ascitic forms of SaI.

■ Graduate Trainee, Research Training Grant CRT5040, Nat. Can. Inst., U.S.P.H.S.

15 *Immunology*

THE USE OF HETEROLOGOUS TRANSPLANTS FOR DETECTION OF ONCOGENIC EFFECT OF ROUS SARCOMA VIRUS. M. Michael Sigel, Thomas M. Scottiy Victor Schuls*, Theodore Burnstein*. University of Miami School of Medicine, Departments of Microbiology and Pathology and Variety Children's Research Foundation.

Rous sarcoma virus (RSV) was shown to induce sarcomatous alterations in the mesenchyme of chick embryo tissue transplanted into the rat brain. When untreated chick embryo mince was implanted into the brains of conditioned rats, one observed the persistence and possible growth of various chick tissue components. The cartilaginous elements were particularly prominent. If such a mince was mixed with the virus of Rous sarcoma and then implanted, the histologic picture of the brain changed to that of sarcomatous growth of the Roustype emerging from normal embryonic tissues. Rats inoculated with RSV alone showed no evidence of tumor. Virus assays showed no evidence of infectious agents at 24 hours of implantation. Thirteen days later there were 4.5 to 5.2 logs of virus in the implanted brains as measured by titration on the CAM. Although some tissue survival was obtained in unconditioned animals, the development of Rous sarcoma in the brain required the conditioning of animals by X-rays and cortisone.

16 *Immunology*

IMMUNOLOGIC RECOGNITION OF TRANSPLANTATION ANTIGENS
J.H. Berrian and C.F. McKhann (intr. by Ray D. Owen)
Naval Medical Research Institute, Bethesda, Md.

Skin homografts between coisogenic sublines of C57BL.10 mice survive 20-40 days in the face of weak gene differences at the H-3 locus and only 9 days when stronger genes at the H-2 locus are involved, reflecting differences in the activity of the corresponding antigens. Where in H-2 combinations all tissues appear to be antigenically equivalent, in H-3, vast differences are found between various tissues. Lymphoid cells given i.p. are totally inactive when H-3 antigens alone are involved. However, when donor lymphoid cells present H-2 with H-3 antigens, immunity is promptly developed against both. Evidently, the prompt response to lymphoid cells is contingent on their recognition per se as being foreign, in a phase preceding the fixation of individual antigens for antibody formation, and the inactivity of H-3 antigens due to their inaccessibility in the cells. In skin homografts, the onset of immunity against H-3 is not accelerated by the presence of H-2 antigens; apparently, immunity is accomplished by liberation and transfer of individual antigens and not by intact cells. Consequently, in skin homografts, H-3 antigens do not trade on the recognition afforded by H-2 antigens and immunization proceeds independently against both.

17 *Immunology*

SERUM ANTIBODIES FOLLOWING HOMOTRANSPLANTATION OF HUMAN CANCER CELL LINES. Miki Aizawa (intr. by A.E.Moore) Sloan-Kettering Institute for Cancer Research, New York City.

Southam and Moore reported that following rejection of subcutaneous homografts of human cancer cell lines (HEp3, HEp2, J-111,etc.) both normal recipients and cancer patients showed accelerated rejection of repeat implants. No serum antibody (Ab) was detected by methods used at that time. The present paper reports success in detection of Ab in these same sera by Boyden's tanned erythrocyte technique using homogenized tissue-cultured cells of the injected types as antigens. Ab was never detected prior to implantation. Ab was found after primary implants in 18 of 21 normal recipients and 10 of 17 cancer patient recipients. After repeat implants 10 of 14 normals and each of 3 patients developed Ab. Ab usually appeared by day 14 and became undetectable (<1:10) again shortly after regression of the implant. Serum Ab persisted longest in some cancer patients and in such cases was concurrent with continuing implant growth. Maximum serum dilution titers were 1:80. There was complete cross-antigenicity between the several cancer cell types used for implantations and antigens, as judged by untreated reagents. Studies with normal cell antigens and with adsorbed sera are still in progress.

18 *Immunology*

THE "R-1 FACTOR" A HISTOCOMPATIBILITY HEMAGGLUTINOGEN IN THE RAT. Arthur E. Bogden* and Paul M. Aptekman. Div. Cancer Immunol., Biochem. Res. Fdn., Newark, Del., and Wistar Inst., Philadelphia, Pa.

Studies of the humoral antibody response in the 10% of randombred Wistar Institute strain rats resistant to implantation of its "native" ascites tumor (AA), revealed a hemagglutinin that reacted with the erythrocytes of 90% of Wistar Institute strain rats. The presence or absence of this isoantigen (termed R-1 factor hemagglutinogen) in the host's tissues determined growth or rejection of intraperitoneal AA tumor implants. A second histocompatibility factor was detected by subcutaneous implantation of tumor ascites and appears to be non-hemagglutinogenic. Several rat strains were screened for the R-1 hemagglutinogen. The inbred P.A. strain was 100% R-1 positive and the inbred Lewis strain was 100% R-1 negative, indicating the homogeneity of inbred systems. Heterogeneity was indicated in the randombred Wistar strain (90% R-1 positive), and in the penbred Chocolate strain (88% R-1 positive). Results of a cross between homozygous R-1 positive and R-1 negative rats indicate that inheritance of this hemagglutinogen is paralleled by susceptibility to intraperitoneal implantation of tumor ascites and follows simple Mendelian laws. The anti-R-1 hemagglutinin induced in R-1 negative animals did not agglutinate erythrocytes suspended in saline.

143. IMMUNE RESPONSE–Vaccines

Immunology

1

DEVELOPMENT OF A FORMALINIZED RIFT VALLEY FEVER VACCINE. R. Randall, C. J. Gibbs, Jr., C. G. Aulisio and L. M. Binn (intr. Geoffrey Edsall.) Walter Reed Army Institute of Research, Washington 12, D. C.

Rift Valley fever (RVF) is a widespread arthropod-borne virus disease of sheep, cattle and man in East, Central and South Africa. In 1951 more than 100,000 sheep and cattle died from RVF in South Africa and there were 20,000 human cases. Live attenuated virus vaccines prepared with neurotropic strains have been widely used in animals but are not acceptable for man, and the viscerotropic virus is highly hazardous to handle in the laboratory. Since a formalin-killed vaccine of this strain was of marginal potency in man, an effective yet safe vaccine made from the viscerotropic strain has been sought. This report concerns development of a safe and effective formalin-killed vaccine. Monkey kidney monolayer cultures in medium 199 are inoculated with viscerotropic strain of RVF. After incubation at 36°C for 96 hours tissue culture fluids containing not less than $10^{7.5}$ mouse LD_{50}/ml are harvested, filtered and formalin added to 1:1000 concentration. Processing and safety testing methods employed resemble those prescribed for production of poliomyelitis vaccine. Vaccinated personnel have neutralizing antibody levels averaging 3.62 logs and have handled infected material without acquiring the disease.

Immunology

2

DEVELOPMENT AND APPLICATIONS OF NEW PARENTERAL ADJUVANTS. III. FACTORS INVOLVED IN ENHANCEMENT OF ANTIGENICITY OF INFLUENZA VIRUS BY HEXADECYLAMINE. K.E. Jensen, A.F. Woodhour*. Chas. Pfizer and Co., Inc., Terre Haute, Indiana.

Results of continuing studies have amplified previous observations that hexadecylamine will increase antibody responses to myxoviruses. Attention was given to physical factors influencing the adjuvant effect and comparisons were made of primary and secondary antibody responses to influenza vaccines injected in animals and human subjects. Vaccines made by saturating HDA with virus are no more antigenic than mixtures of the components. Although effectiveness of the booster dose is not enhanced by this adjuvant, secondary antibody titers are influenced by the vehicle used for sensitization and primary levels obtained. HDA combines more readily with influenza virus than with egg derivatives thus allowing considerable purification and concentration of vaccine.

Immunology

3

DEVELOPMENT AND APPLICATIONS OF NEW PARENTERAL ADJUVANTS. IV. INFLUENZA VACCINE WITH HDA IN OIL EMULSIONS. A.F. Woodhour*, J. Warren and K.E. Jensen. Chas. Pfizer and Co., Inc., Terre Haute, Indiana.

Remarkably potent vaccine can be prepared using influenza viruses complexed to hexadecylamine and emulsified with several different oils and surfactants. Combinations studied have included mineral and vegetable oils, Arlacel and Tween, and a thickener to produce both water-in-oil and oil-in-water emulsions. A single injection of animals with vaccine incorporated in certain of these formulations produces an excellent, prolonged response with titers of antibody being enhanced several hundredfold greater than that obtained with control vaccines. Results of preliminary toxicity studies and tissue reactions with the newer combinations indicate acceptability of these complexes adequate to consider trials in man.

144. LIPIDS

Biochemistry

1

LIPIDE COMPOSITION OF NORMAL HUMAN BONE MARROW. Djahanguir K. Abadi*, James C. Mathies, and Paul K. Lund*, Pacific Northwest Research Foundation, Inc., and The Swedish Hospital, Seattle, Wash.

This study was initiated to provide a background for an evaluation of the lipide composition of xanthomatous tissues of bones and joints. Histologically normal marrows were obtained post-mortem from the femurs of 12 subjects (ages 1-78 years). The total lipide, extracted by the procedure of Folch, et al (J. Biol. Chem. 226, 479 (1957)), represented 43-84% of the tissue wet weight, and contained 97-99% of neutral lipide and less than 3% of phospholipide. This extract was then fractionated on silicic acid by the procedure of Barron and Hanahan (J. Biol. Chem. 231, 493 (1958)). Hydrocarbons, cholesterol esters, monoglycerides, and diglycerides were not present in detectable amounts. Cholesterol and free fatty acids were present at the less than 1% level. The predominant fraction consisted of neutral triglycerides containing the long chain fatty acids with an iodine number of 69-81. The fraction of the total neutral lipide present as triglyceride was constant at 96-98% in all of the specimens thus exhibiting no variation with age. Preliminary results on the lipides of selected xanthomatous diseases of bone and joints will also be presented. (Supported by Grant A-2340, Natl. Institutes of Health, PHS.).

Biochemistry

2

THE PAPER CHROMATOGRAPHY OF CEREBROSIDES AND SULFATIDES OF BRAIN TISSUE. C. B. Scrignar and V. J. Ferrans (intr. by E. Farber.) Departments of Medicine and Anatomy, Tulane Univ. School of Med., New Orleans, La.

A method for the separation of CNS lipids has been devised utilizing silicic acid impregnated paper and a solvent system consisting of diisobutylketone, pyridine, water (55:41:4). 10-50 ul. of brain lipid extract were chromatogrammed at 25° C for 2-6 hours with greater resolution occurring in the longer runs. The lipid extracts of white matter from monkey, dog, beef and whole brain from rat were compared with a phrenosin preparation obtained from E. Klenk, a sulfatide sample supplied by J. Folch, and cerebroside and sulfatide preparations made in our laboratory. By various spot tests the following lipids were identified in decreasing order of mobility: cerebrosides, cephalins, sulfatide, lecithin, and sphingomyelin. After staining with Rhodamin 6G, the chromatograms were examined under ultraviolet light. Sphingomyelin and cerebrosides fluoresced yellow, the sulfatide a characteristic orange and the lecithin and cephalin plasmalogens purple. (Supported by USPHS grant HTS 5133.)

Biochemistry

3

SEPARATION OF DIFFERENT GANGLIOSIDIC COMPONENTS OF BEEF BRAIN GANGLIOSIDE. H. Weicker*, J. A. Dain*, G. Schmidt, S. J. Thannhauser. Boston Dispensary and Department of Biochemistry Tufts Univ. School of Medicine, Boston, Massachusetts. Ganglioside prepared according to J. Folch and to S. Bogosch is chromatographed by adsorption on thin layer silica gel on glass plates. Solvent: propanol/ N NH_4OH/ H_2O/ 6:2:1 V/V/V. Three separate spots appear on the plates. The same system is used for column chromatography. Three main fractions consisting of gangliosidic components adsorbed to ammonium silicate are eluated. These three lyophilized fractions were treated with chloroform:methanol 2:1 and finally extracted with hot 95% ethanol. The remaining residue is ammonium silicates. The dried substances (I, II, III) of the chloroform methanol solutions contain different amounts of neuraminic acid (NA) I 26%, II 26%, III 18%. Hexosamin I 7%, II 5%, III 4%. Tot. hexoses I 18%, II 15%, III 13%. These fractions are in contrast to the original ganglioside only colloidal water soluble. The dried substances of the ethanol solution contain in two fractions considerable amounts of complete water soluble material (I_w, III_w). III_w crystalized in rosettes and melted with decomposition at 190/191° NA 25%, hexosamin 7% and tot. hexoses 27%. C51.8%, H 8.3%, N 3.2%. I_w inhibited live Influenza A virus agglutination. III_w shows no inhibition (M. A. Madoff). The isolation of diff. gangliosidic components of ganglioside is described.

XUM

4 *Biochemistry*

SEPARATION OF LIPID CLASSES BY CHROMATOGRAPHY ON FLORISIL. K. K. Carroll. Collip Medical Research Laboratory, University of Western Ontario, London, Ontario, Canada.

Silicic acid chromatography has been widely used to separate different lipid classes such as cholesterol esters, triglycerides, phospholipids, etc. Chromatography on Florisil (activated magnesium silicate, 60/100 mesh) has been employed by Radin and associates for purification of cerebrosides and separation of straight-chain from hydroxylated fatty acid esters (J.Biol.Chem. 219:977, 1956, J.Lipid Chem. 1:72, 1959). The present work indicates that the adsorption properties of Florisil for neutral lipids are similar to those of silicic acid. Cholesterol esters, triglycerides, cholesterol, diglycerides and monoglycerides may be readily and completely separated by elution from a Florisil column with purified Skellysolve B (mainly n-hexane) containing increasing proportions of diethyl ether. Yields with model compounds have been nearly quantitative. Free fatty acids and phospholipids are retained more strongly on Florisil than on silicic acid. Flow rate is not a problem with Florisil columns and in our experiments the Florisil was not subjected to preliminary washing or drying procedures. Florisil thus appears to offer advantages over silicic acid for the separation of lipid classes.

5 *Biochemistry*

PARALLELS BETWEEN ENZYMATIC SULFURYLATION OF N-ACETYL SPHINGOSINE AND CHLORAMPHENICOL. Irving H. Goldberg (intr. by John D. Gregory). The Rockefeller Institute, New York, N. Y.

Purified fractions from rat liver supernatant have been shown to transfer $S^{35}O_4$ from PAPS^{35} to lipid (I. H. Goldberg and A. Delbrück, Federation Proc., 18, 235, 1959). This transfer is dependent upon the addition of a crude mixture of the stereoisomers of N-acetyl sphingosine. Evidence has been obtained suggesting direct sulfurylation of the sphingosine derivative. Because of striking structural similarities between N-acetyl sphingosine and chloramphenicol, sulfurylation of the latter was attempted with the same enzyme preparation. Natural chloramphenicol (D-threo) accepted $S^{35}O_4$ from PAPS^{35}. The most active isomer, however, was the D-erythro which was about 20 times as active as the D-threo. The enzymatically sulfurylated compound was identical electrophoretically and chromatographically with chemically synthesized chloramphenicol monosulfate. Structural and electronic requirements for enzymatic sulfurylation of derivatives of chloramphenicol and other alcohols with aromatic substituents have been studied. The enzymatic system appears to be distinct from the phenol and steroid sulfokinases.

6 *Biochemistry*

THE BIOSYNTHESIS OF UBIQUINONE (COENZYME Q) IN RATS. Harry Rudney and Takashi Sugimura*. Dept. of Biochemistry, Western Reserve U., Cleveland, Ohio.

Previous work has shown that acetate is quickly incorporated into the side chain of ubiquinone (Fed. Proc. 18, 214, 1959). The origin of the benzoquinone ring is still uncertain. 200 μC C^{14} formate were administered over 4 days to rats, to determine if the methoxyl group on the benzoquinone nucleus became labeled. C^{14} could be barely detected in ubiquinone 45 (?) isolated from liver, although other fractions of the lipid extract were highly labeled. C^{14} methoxyl labeled ubiquinone 30 obtained from yeast incubated with C^{14}-formate was emulsified with rat serum and injected intravenously into rats. After 24 hrs the livers from the rats were analyzed. It was found that ubiquinone 30 and 45 (?) were present in the mitochondrial fraction. However only ubiquinone 30 was labeled, coenzyme Q_9 was unlabeled. A small amount of radioactivity was detected in another fraction which appeared to be a breakdown product of ubiquinone 30. These results show that the methoxyl groups of the benzoquinone nucleus are at best very slowly synthesized in the rat and that ubiquinone C-30 isoprenologues are not readily converted to higher isoprenologues.

7 *Biochemistry*

ISOLATION AND IMMUNOCHEMICAL ASSAY OF CEREBRAL MYELIN. Frank H. Hulcher and Miles R. Cooper.[1] (intr. by William A. Wolff) Dept. of Biochem. and Neurology Section, Bowman Gray School of Medicine, Wake Forest College, Winston-Salem, N. C.

White matter was separated from the internal capsule of bovine brain, homogenized in 0.5M sucrose solution and fractionated by differential centrifugation, the myelin fraction being characterized by determining the ratios cholesterol:phosphorus: nitrogen, and by staining with sudan black B. The fraction was freed from water-soluble materials by extensive washing. Infrared spectrum analysis indicated that the fraction was relatively free of extraneous materials. When this fraction with added Freund adjuvant was injected into rabbits, antibodies, exhibiting both agglutinin and precipitin reactions, were formed in one month. Control sera did not react with the antigen. The precipitate obtained in the in vitro reaction was analyzed for cholesterol and nitrogen. For the development of a quantitative assay procedure of the precipitin reaction the myelin fraction was solubilized by anionic detergents. Sodium alkyl sulfate (P&G) proved the most effective; Tweens 20 and 80 were ineffective. It is believed that this immunochemical technique offers possible advantages for the study and quantitative assay of myelin. (Supported in part by the National Multiple Sclerosis Society and [1] Student Fellowship Fund, U.S. Public Health Service.)

8 *Biochemistry*

BIOSYNTHESIS OF COENZYME Q_9 IN THE RAT. Robert E. Olson, G. Hossein Dialameh, * and Ronald Bentley. Dept. of Biochem. and Nutr., Univ. of Pittsburgh, Pittsburgh, Pa.

Both coenzyme Q_9 and Q_{10} have been identified in rat tissues by paper chromatography (Linn et al, J.A.C.S., 81, 4007 (1959)). The incorporation of acetate-1-C^{14} (Fed. Proc., 18, 214 (1959)) and mevalonic acid-2-C^{14} (Gloor and Wiss, Arch. Biochem. & Biophys., 83, 216 (1959)) into coenzyme Q rechromatographed or recrystallized to constant specific activity with carrier coenzyme Q_{10} has been reported. In view of the difficulty in separating these homologues, the incorporation of IP acetate-1-C^{14} into hepatic coenzyme Q_9 and Q_{10} in the rat was restudied. It was found that 80-90% of the hepatic Q in the rat on both chow and synthetic Q-free diets was Q_9 and the remainder Q_{10}. Essentially all of the radioactivity in coenzyme Q (.01% dose) was associated with Q_9. Pure Q_9 was isolated from normal rat liver and characterized by m.p., u.v. spectrum, C,H, and CH_3O- analyses and R_f. Since aromatic biosynthesis is unknown in the rat, the incorporation of radioactivity from possible precursors of the benzoquinone moiety of Q_9 has been studied. The ratio of the radioactivities of hepatic Q_9:cholesterol in seven experiments was 2.7 with phenylalanine-U-C^{14} and 1.8 with acetate-1-C^{14}. Degradation of the two isolated Q_9s has also revealed differences in the pattern of labeling.

9 *Biochemistry*

INCORPORATION OF SERINE-3C^{14} RADIOACTIVITY INTO CEREBRAL GLYCOLIPIDS. Luis Garcia* and Robert Main Burton. Dept. of Pharm. and the Beaumont-May Inst. of Neurology, Washington Univ. Sch. of Medicine, St. Louis.

Glucose and galactose are incorporated into the sphingosine containing glycolipids of brain tissue with maximal rates occurring at ages characteristic of the type of glycolipid. In white male rats, galactose-C^{14} is found in the ganglioside fraction at about 6-10 days post-partum (Mandelstam and Burton, Fed. Proc. 18, 1959) and in the cerebroside fraction at 10-18 days (Burton, Sodd, and Brady, J. Biol. Chem. 233, 1053, 1958). Zabin and Mead (J. Biol. Chem. 205, 271, 1953) and Brady and Koval (J. Biol. Chem. 233, 26, 1958) have shown that serine is a precursor of sphingosine. We have found that DL-serine-3C^{14} radioactivity was incorporated, in vivo, into both the cerebroside and ganglioside fractions of rat brain tissue. The cerebroside fraction was labeled with carbon-^{14} at a maximal rate in rats 14 to 18 days post-partum. Serine radioactivity was found in the ganglioside fraction of brain tissue with animals as young as 5 days. Maximum rate of incorporation into the ganglioside fraction occurred with rats older than 14 days. A comparison of the incorporation of serine and galactose into the glycolipids at various ages will be discussed. This work was supported by NINDB Grant B-1575C1 and NSF Grant G9867.

10 *Pharmacology*

THE EFFECTS OF d-TRIIODOTHYRONINE ON SERUM CHOLESTEROL AND THYROID FUNCTION IN HYPERCHOLESTEREMIC PATIENTS. A.P. Crosley, Jr., F. Alexander * and W.H. Strickland *. Mercy-Douglass Hospital, Philadelphia, Penna.

Previous work in rats suggested that d-triiodothyronine possessed about 0.1 the metabolic stimulating activity of the levo form while retaining equipotency with regard to its ability to lower serum cholesterol. Accordingly this agent, in a dosage of 1.0 mg./day, was administered to 4 hypercholesteremic patients for 4 weeks following a control period of 4 weeks. Simultaneously an additional 4 patients were studied according to this protocol except that a placebo was used during the drug phase. In all patients serum cholesterol determinations were performed at weekly intervals while estimations of BMR, PBI and 24 hour thyroid I 131 uptakes were made before and at the completion of the treatment period. During the control phase the difference of the mean weekly serum cholesterol values of the placebo group differed from those of the patients who were to receive the active agent by +3 to - 36 mg. %. During the placebo - treatment period the range of such differences was - 21 to - 146 mg.%. The determinations of thyroid function showed no consistent alterations. These data indicate that d-T_3 in an effective agent in the lowering of serum cholesterol in man while at the same time producing negligble effects on the BMR, PBI and thyroid I ^{131} uptake.

11 *Pharmacology*

ON THE EFFECT OF ARACHIDONIC ACID ON SERUM LIPIDS IN MAN. Martin M. Nothman and Samuel Proger*, New England Center Hospital and Tufts University School of Med., Boston, Mass.

The ability of highly unsaturated oils, such as safflower, corn or cottonseed oil to lowering serum cholesterol has been attributed to linoleic acid, because these oils contain 40-60 % linoleic acid and hardly any of the other highly unsaturated fatty acids. To determine the effectiveness in decreasing serum lipid fat constituents of each of the three so called essential fatty acids, the serum was examined after ingestion of linoleic, linolenic and arachidonic acid separately and of a stable mixture of these three unsaturated fatty acids. Purified linoleic acid alone has some lowering effect on serum lipids. Linolenic acid alone has no recognizable effect. When given with linoleic acid, the effect of linoleic acid is increased. Addition of arachidonic acid, even in small amounts, increases the effect further. The greatest fall in serum lipids occurs when large quantities of arachidonic acids are ingested. In cases where cholesterol has been as high as 800 mg %, the mixture of linoleic, linolenic and small amounts of arachidonic acid, lowered the value to 400-500 mg % and kept it stable at that level. 25 gm of arachidonic acid daily decreased the value to 200-250 mg %. Even with these additional quantities of unsaturated fatty acids, the weight of the individuals hardly changed.

12 *Nutrition*

A MICRO-METHOD FOR SEPARATION OF LIPIDS BY SILICIC ACID CHROMATOGRAPHY. Elaine W. Lis and Joan Tinoco (intr. by Ruth Okey) Dept. of Nutr. & Home Econ., Univ. of Calif., Berkeley, Calif.

Two to 10 mg of lipid are separated on silicic acid columns into four fractions: fraction I, cholesterol esters; II, triglycerides; III, free cholesterol, free fatty acids; IV, phospholipids. Ten columns are operated at one time using a glass manifold connected to a tank of nitrogen and to ten glass stoppered outlets which lead to the columns. One gram of specially prepared silicic acid is packed into each glass column, (8 mm by 140 mm fused to glass tubing 20 mm by 110 mm) washed successively with methanol, acetone, ethyl and petroleum ether, all purified. The lipid is added in 100 to 200 ul of petroleum ether and rinsed onto the column with two 200 ul petroleum ether rinses. The fractions are eluted with a modification of the solvent system of Fillerup and Mead (Proc. Soc. Exp. Biol. Med. 83:574, 1953) using 15 ml of each mixture; fraction I with 1% ethyl ether in petroleum ether, II with 4% ethyl ether in petroleum ether, III with 50% ethyl ether in petroleum ether, IV with methanol. The nitrogen flow is adjusted so that each fraction is eluted in approximately 30 minutes. The solvent is evaporated, the lipid saponified where necessary, and the fatty acid moiety methylated and separated by gas liquid chromatography. (Supported in part by grant H-2152, PHS.)

13 *Nutrition*

EFFECT OF STERCULIA FOETIDA OIL ON YOLK WEIGHT AND WATER UPTAKE OF THE HEN EGG. D. L. Schneider*, A. R. Doberenz*, A. A. Kurnick*, M. G. Vavich*, and A. R. Kemmerer. Depts. of Agric. Biochem. and Poul. Sci., Univ. of Arizona, Tucson.

W.L. hens maintained on a practical type basal diet were supplemented daily with 0.3 g of commercial corn oil or 0.15 g S. foetida oil-0.15 g corn oil via gelatin capsule. Starting with the seventh day of supplementation, eggs were stored at 7°C either overnight or for one month. Water uptake was measured as the increase in weight of the separated yolk at 37°C when placed in a beaker of deionized water for two hours. When the eggs were stored overnight, no significant difference was found between the two treatments. Average yolk weight and water uptake were 18.61±1.07 and 0.95±0.29 g for the corn oil supplemented lot and 19.24±0.09 and 1.03±0.09 g for the S. foetida oil treated lot. However, after storage of one month highly significant differences ($P>0.001$) were found. The yolk weight and water uptake were 19.36±1.36 and 6.13±2.07 g respectively for the corn oil treated lot while the S. foetida oil treated lot had a yolk weight of 23.35±1.74 g and water uptake of 11.12±4.64 g. This represents a 20.6% increase in yolk weight and an 81.4% increase in the ability of the S. foetida supplemented eggs to take up water over those supplemented with corn oil. (Supported by a grant from Mead Johnson and Co., Evansville, Ind.)

145. LIPIDS—Fats and Fatty Acids

1 *Biochemistry*

POLYUNSATURATED FATTY ACIDS IN NORMAL GROWING RATS. John C. Kirschman* and John G. Coniglio. Dept. of Biochem., Vanderbilt Univ. School of Med., Nashville, Tenn.

The concentrations of polyunsaturated fatty acids, measured spectrophotometrically after alkaline isomerization, were determined in various organs of weanling, 3-month and 6-month old Sprague-Dawley rats maintained on a purified diet containing 18% casein and 25% Crisco. Significant findings included the absence of dienoic and trienoic in the presence of large amounts of tetraenoic and hexaenoic acids (15-20 mg./100 mg. TFA) in brains of animals of all groups. High levels of tetraenes were found in heart, liver, kidney and spleen (20-28 mg./100 mg. TFA). Pentaene concentration was highest in testicular tissue (about 20 mg./100 mg. TFA). Similar values were found for the polyene levels of the two sexes within any age group. There were no marked differences in polyene levels of weanling compared to 6-month old rats, but some decreased concentrations were observed in 3-month old animals. The data show growth did not cause a change in concentrations of polyenoic acids in these animals. (Supported by AEC Contract No. AT-(40-1)-1033 and a grant from the Nutrition Foundation).

2 *Biochemistry*

THE EFFECT OF EPINEPHRINE ON THE LIPOLYTIC ACTIVITY OF ADIPOSE TISSUE. Martin A. Rizack (intr. by R. M. Archibald.) Rockefeller Institute, New York.

Paired epididymal fat pads were removed from rats pretreated with pentobarbital and hexamethonium and placed in Krebs-Ringer-Phosphate solution for incubation. Epinephrine was added to the incubation medium of one of each pair of fat pads in a concentration of 0.16 μgm/ml. Following incubation fat pads were homogenized in sucrose and lipolytic activity determined by measuring the fatty acid released when the homogenate was incubated with a coconut oil emulsion ("Ediol") in phosphate buffer at pH 6.8 containing 5% albumin. After three hours of incubation the lipolytic activity of fat pads exposed to epinephrine was more than twice that of their controls. When glucose in a concentration of 5 mM was added to the incubation medium containing epinephrine there was an increase in lipolytic activity throughout the 3-hour period of incubation. In the absence of glucose, activity rose during the first half hour and fell gradually thereafter. Pretreatment of animals with pentobarbital and hexamethonium increased the uniformity of epinephrine response. Tissues removed from starved animals demonstrated a diminished epinephrine effect. Lipolytic activity sedimented completely from 0.25 M sucrose centrifuged at 105,000 G for 12 hours.

XUM

3 *Pathology*

RELATION OF LINOLEIC ACID AND CHAIN LENGTH OF TRIGLYCERIDES TO NEUTRAL FAT DEPOSITION. Hans Kaunitz, C. A. Slanetz*, R. E. Johnson*, and V. K. Babayan*. Dept. Path. and Inst. Comp. Med. Columbia Univ., New York and E. F. Drew & Co., Boonton, N. J.

Matching groups of 16 linoleic acid-depleted rats were fed, for 84 days, purified diets containing no fat (FF) or 20% saturated long chain (C_{14-18}) or medium chain (C_{6-12}) triglycerides (LCT, MCT). The rations were supplemented with no, .1%, or 2% linoleic acid (LA). Testicular fat bodies were weighed because they are an expression of total neutral fat (Hausberger, Z. exp Med. 102: 169, 1937). Their weight was expressed as percentage of deviation from that of normal rats of identical body weight. Data are summarized in the table. ± values are standard errors.

	0% LA		.1% LA		2% LA
Fat-free	+10±7.7		-5±4.9	P<.01	-26±4.5
MCT	+28±5.8	P<.01	+2±5.7	P<.01	-23±5.0
LCT	+58±6.1		+50±6.3	P<.01	-9±5.5

Neutral fat deposition was significantly higher in all LCT groups than in corresponding ones fed MCT or FF. Smaller amounts of LA exerted a greater effect with MCT than with LCT, suggesting lower LA requirements with MCT. The reduction of fat deposition with MCT may have been partly responsible for the observation (J.Nutr. 64: 513, 1958) that humans fed reducing diets with MCT as the fat lost weight more rapidly than with equal amounts of natural fats.

4 *Nutrition*

POSITION OF LINOLEIC ACID IN THE TRIGLYCERIDES OF MOUSE DEPOT FAT. S. B. Tove and F. H. Smith*. Nutrition Sec., North Carolina State College, Raleigh.

Linoleic acid levels varying from 15-66% of the depot fat of mice were produced by feeding graded levels of safflower oil. The fatty acid composition of the total triglycerides and of the 2-monoglycerides, isolated after partial hydrolysis by pancreatic lipase, was determined by gas chromatography. A plot of the depot fat linoleate against dietary linoleate showed 2 slopes. Coincident with the 2 slopes were 2 patterns of fatty acid substitution. The linoleate content of the 2 position tended to remain constant at about 15% of the total fatty acids, possibly accounting for the previously observed (Tove and Smith, Arch. Biochem. Biophys., in press) slow depletion of linoleate occurring at that level. Most of the linoleate was esterified to the 1,3 positions of the glycerol. The 2 slopes were observed for the alpha esterified linoleate with the change in slope occurring at a level of 33% linoleate. These results indicate that there is a selective deposition of linoleate in the 2 position of the glyceride until a level of about 15% is reached after which deposition in the alpha positions predominate. It is possible that the 2 different patterns of fatty acid substitution are associated with the 2 alpha positions of the glyceride. (Supported in part by grant from U. S. Public Health Service.)

5 *Nutrition*

METABOLIC CHANGES IN TOCOPHEROL DEFICIENT RATS. B. Century* and M. K. Horwitt, Mendel Research Lab., Elgin State Hospital, Elgin, Illinois.

Tocopherol deficiency in rats, as measured by severity of creatinuria, was related to the degree of unsaturation of the ingested lipids. Using fats stripped of tocopherol, significant elevation of creatine excretion was observed after 11 weeks on a 7% cod liver oil diet, 15 weeks with 7% linseed oil or 15% corn oil, and 19 weeks with 15% olive oil. Highest creatine to creatinine ratios observed were 1.42, 1.40, 1.56, and 0.56, respectively, for these diets. Increased creatinuria was not seen in rats given 15% coconut oil or fat-free (actually 0.2% corn oil) diets for 29 weeks. Rats given 15% corn oil for 40 to 52 weeks required 0.4 mg. d-α-tocopherol acetate/week for optimal growth and prevention of increased nucleic acid phosphorous in skeletal muscle, and up to 0.7 mg/week to prevent increased creatinuria and elevated labeling of muscle nucleic acids with I. P. injected formate-C_{14}. These metabolic changes may be secondary to the deteriorations in tissue integrity due to loss of other vital functions.

6 *Nutrition*

SERUM LIPID PATTERNS IN MOTHERS AND THEIR NEWBORN INFANTS. C.W. Woodruff and J.G. Coniglio, School of Medicine, Vanderbilt University, Nashville, Tennessee.

Total fatty acids, polyunsaturated fatty acids, phospholipids, free and total cholesterol, vitamin A, tocopherol and carotene were measured in 88 mothers at term and in their infants before the first feeding. Maternal values fell in a higher range for all the lipids studied. Total fatty acids averaged 402 ± 15 mg. per cent in the mothers and 208 ± 8 in the infants. The pattern of polyunsaturated fatty acid was different in two groups. The mothers had 25.7 ± 0.6 per cent dienoic and 5.98 ± .4 per cent tetraenoic fatty acids. The infants had 7.4 ± .4 per cent dienoic and 11.8 ± .5 per cent tetraenoic fatty acids. The concentrations of penta- and hexaenoic acid were also higher in the newborns. Trienoic acids were not significantly different. Fatty acid composition of the sera of some newborns and mothers has been studied by gas-liquid chromatography. The most consistent finding was a relatively higher concentration of palmitoleic acid in the serum of newborn infants. The data suggest several hypotheses concerning placental transfer and fatty acid metabolism in the fetus. (Supported by a grant from the Nutrition Foundation

7 *Nutrition*

INHIBITION OF CHICKEN GROWTH BY CHOLESTEROL AND SATURATED FATTY ACIDS (SFA) AND REVERSAL BY A SOURCE OF UNSATURATED FATTY ACIDS (UFA). L. J. Machlin and R. S. Gordon*, Monsanto Chemical Company, St. Louis, Mo.

It has been reported that chickens fed fat-free diets for 8 weeks grow normally. In the present studies a growth response to a source of UFA was observed at 2 weeks of age with chickens fed a basal purified diet containing 1% methyl myristate, 21% casein, 5% glutamic acid and 2.0% glycine. The 7-27 day gains (in gm.) observed with this diet and supplements were: basal, 259; 2% UFA (safflower oil added isocalorically), 301; 8% SFA (3% methyl myristate+5% methyl laurate), 217; 8% SFA+2% UFA, 285; 2% cholesterol, 252; 2% cholesterol+2% UFA, 306; 8% SFA+2% cholesterol, 185; 8% SFA+2%UFA, 279. Dietary cholesterol increased plasma cholesterol from 183 mg% to 361 mg% on chickens fed the basal diet, and from 165 mg% to 1316 mg% in chickens fed 8% SFA. It is concluded that UFA can reverse the inhibition resulting from dietary SFA or from cholesterol fed with dietary SFA. It is probable that SFA and cholesterol increases the requirement for essential fatty acids.

8 *Nutrition*

THE EFFECT OF DIETARY FAT ON THE FATTY ACID COMPOSITION OF BRAIN AND LIVER MITOCHONDRIA. G. J. Marco*, L. J. Machlin and R. S. Gordon*, Monsanto Chemical Company, St. Louis, Mo.

Mitochondrial fractions were prepared by differential centrifugation from liver and cerebellum of chicks fed fat-free diets or diets containing 12% of tallow, linseed oil, a 71% linoleate oil, or oleic acid. Crude homogenates of pooled tissues or mitochondria prepared from the homogenates were saponified. The fatty acids were esterified and analyzed by gas chromatography. The mitochondrial fatty acid spectrum was similar to its respective homogenate. Liver and cerebellum samples from chicks fed the high linoleate diet contained 2 to 3 times the linoleic acid concentration of other groups. The samples from chicks fed linseed oil contained 1.5 to 4 times the linolenic acid concentration of other groups. Oleic acid fed chicks showed increased oleic acid content in the liver. The structural fatty acids of rapidly growing chicks appear to be greatly affected by dietary fat. This supports the hypothesis that a high linoleic acid content of cerebellum is necessary for development of encephalomalacia.

146. LIPIDS—Fats and Fatty Acids—Metabolism

/ *Physiology*

A SURVEY OF THE EFFECTS OF CARNITINE ON FATTY ACID OXIDATION BY TISSUE PREPARATIONS. Irving Fritz and Eli Kaplan*. Dept. of Physiology, Univ. of Michigan, Ann Arbor, Michigan.

Carnitine addition is followed by increased conversion of carboxyl-labeled myristate, palmitate, stearate, and oleate to $C^{14}O_2$ by liver and heart muscle slices, whole homogenates and mitochondria. The oxidation of stearate-2-C^{14} is enhanced by carnitine as much as is the degradation of stearate-1-C^{14}. Levels of endogenous CoA in heart muscle mitochondria do not limit the rate of long-chain fatty acid oxidation unless carnitine is present at which time maximal effects are dependent upon added CoA. Conversion of carboxyl-labeled short-chain fatty acids by heart muscle mitochondria is not accelerated by carnitine. Absolute oxidation rate of long-chain fatty acids is much slower than that of short-chain fatty acids. Of the tissues examined, heart muscle gave the largest responses to carnitine when added albumin concentrations were kept low, whereas other tissues also showed pronounced increases in long-chain fatty acid oxidation in the presence of carnitine at high albumin levels. Carnitine-responsive tissues thus far examined include skeletal muscle, liver, intestine, kidney, and brain, although the response by the last two organs is small. Of a wide variety of carnitine and choline derivatives tested, only acetyl carnitine has been found to have similar properties to carnitine in the heart muscle system.

2 *Physiology*

ENDOCRINE CONTROL OF PLASMA LIPIDS. Edward Paloyan*, Paul V. Harper and Hannah B. Watkins*. Dept. of Surg., The University of Chicago, Chicago, Illinois.

The body and the tail of the pancreas of the dog have been shown by Bencosme and Liepa (Endocrin. 57:588, 1955) to contain α-cells, while in the uncinate process and the part of the gland drained by the duct of Wirsung, islets are largely devoid of these cells. We have confirmed these observations. In addition, surgical removal of the portions of the gland containing α-cells results in a marked hyperlipemia when a small remnant of pancreas is left to supply pancreatic juice to the bowel. No such hyperlipemia occurs when the remnant contains a significant number of α-cells. These phenomena were demonstrated in 18 dogs. In 6 of these the hyperlipemia was abolished by the intramuscular injection of 5 to 10 mg. of glucagon daily. This effect persisted for 1 to 2 weeks after the administration of glucagon ceased. If we assume that the α-cells are the source of glucagon, as other investigators have suggested, then we must conclude that no significant numbers of these cells exist outside the pancreas in the dog.

(Supported by USPH research grant A-3142. Crystalline glucagon supplied by Eli Lilly and Co.)

3 *Physiology*

EFFECTS OF DIETARY LIPID ON INCORPORATION OF C-14^{14} ACETATE INTO LIVER FATTY ACIDS. J.D. Evans and R.C. Baldridge. Temple Univ. Sch. of Medicine, Philadelphia, Pa.

Adult rats fed purified diets which contained 2% cholesterol and 20% of either olive or safflower oil were injected intraperitoneally with 30 μC of 2-C^{14} sodium acetate and killed 1, 2 or 4 hours later. Similarly fed rats were each injected with a total of 60 μC of C^{14} acetate (20 μC at 0, 24, and 48 hours) and killed 72 hours after the initial dose. Liver lipids were extracted and separated by chromatography on silicic acid into hydrocarbons, sterol esters(SE), glycerides (G), free sterols and phospholipids (PL); these were then separated further into fatty acids (FA) and unsaponifiable material and counted with a liquid scintillation counter. The C^{14} of all liver FA from rats fed mainly trilinolein (in safflower oil) decreased more rapidly with time than did the C^{14} of liver FA from those fed mainly triolein (in olive oil). It thus appears that ingestion of increased amounts of linoleic acid is followed by an increased rate of degradation (or otherwise removal) of liver fatty acids. This is of especial interest as others have reported an enhancement of cholesterol synthesis and degradation in tissues of linoleic acid-fed animals. Spectrophotometric analysis revealed considerable differences in composition of FA of SE and FA of G but not FA of PL of olive and safflower oil-fed rats. (Supported by grants from USPHS H2175 and A650)

4 *Physiology*

THE FATE OF ADMINISTERED TRITIATED CARNITINE IN THE DOG. Kenneth T. N. Yue* and Irving B. Fritz. Dept. Physiology, University of Michigan, Ann Arbor. (Intr. by H.W. Davenport)

Purified H^3-carnitine was injected intravenously into anesthetized dogs, after which blood and urine samples were collected. Activity disappeared from plasma quite rapidly. In a seven hour period, about 41% of administered carnitine was excreted in the urine, and approximately 0.6% remained in blood. The clearance of H^3-carnitine for both kidneys was approximately 31 ml/min in a 17 kg dog. In a separate experiment employing the stop-flow technique of Malvin et al (Am. J. Physiol., 194: 135, 1958), it was shown that carnitine was apparently not reabsorbed by the kidney. Approximately 1/3 of the injected label was found in trichloroacetic acid soluble fractions of various tissues, with the largest amount (29%) in muscle, and highest concentrations in liver. The activity per g wet weight was greater than that of plasma by 15-fold in liver and 5-fold in skeletal and cardiac muscle. Inappreciable activity appeared in brain, and no tritium was detected in red blood cells. No tritium was present in water distilled from various fractions, indicating a lack of degradation of administered carnitine. About 17% of injected label was recovered in hot 95% alcohol extracts of TCA washed residual tissues. The composition of the tritium-containing materials is under investigation.

5 *Physiology*

THE LIPOPROTEIN LIPASE OF RAT HEART. G. L. Feldman (intr. by L. Kraintz). The Methodist Hospital and Baylor Univ. College of Medicine, Houston, Texas.

There is a growing body of evidence which suggests that lipoprotein lipase is the factor responsible for the clearing of postalimentary lipemia. The characteristic lactescence of lipemic serum is produced by the presence of chylomicrons. Clearing involves a degradation of chylomicrons to lipoproteins of higher density by the enzymatic hydrolysis of the triglyceride moiety by lipoprotein lipase. To facilitate the study of this phenomenon, a manometric assay for lipoprotein lipase was developed. Whole homogenates of rat heart exhibited a very marked requirement for heparin which was not observed with cell-free, water extracts. The prolonged administration of heparin resulted in a very marked decrease in the lipoprotein lipase activity. When safflower oil, butterfat and coconut oil were used as substrates it was found that the enzyme activity was inversely proportional to the iodine number and the average fatty acid chain length. Similar data was obtained with pure triglycerides. It is concluded that heparin promotes the release of the enzyme rather than function as a cofactor and that there is a definite relationship between triglyceride structure and enzyme activity. (Supported by Grant no. 603-5-60 from the Houston Heart Association.)

6 *Physiology*

FATTY ACID MOBILIZATION DURING PREGNANCY IN THE RAT. E. Knobil and J.B. Josimovich*. Dept. of Physiology, Harvard Medical School, Boston, Mass.

We have previously reported that the last 3 days of pregnancy in the rat are accompanied by a striking hyperlipemia characterized by a rise in the plasma concentration of total lipids, cholesterol and phospholipids. Maximal concentrations of these components are reached at parturition with a rapid decline thereafter. The plasma concentration of non-esterified fatty acids (NEFA) during pregnancy has now been investigated as well as the release of NEFA from mesometrial adipose tissue in vitro. It has been found that plasma NEFA concentrations are elevated during the latter part of pregnancy and that NEFA release from adipose tissue is increased during pregnancy. These findings suggest increased fatty acid mobilization from peripheral fat stores during late pregnancy. This phenomenon is associated with a significant reduction in fasting blood sugar concentrations. The physiological significance of these findings will be discussed.

(Supported by PHS Grant A-503).

7 *Biochemistry*

METABOLISM OF BRAIN AND LIVER SULFATIDES. Jerome Bakke* and W. E. Cornatzer, Dept. of Biochem., Univ. of N. Dak. School of Med., Grand Forks, N. Dak.

The lipides were extracted and purified by the method of Folch (J. Biol. Chem. 226, 497, 1957), oxidized by a Carius technique using aqua-regia, the excess of oxidizing agent removed and sulfur assayed by method of Archer (Analyst 81, 181, 1956). The lipide sulfur level (μgS/gm fresh tissue and μgS/mg N) in liver of male albino rat or the rate of sulfatide synthesis from S^{35}-Methionine does not change significantly with age (22 to 180 days) while the brain sulfatide accumulates over the 22 to 180 day age span with the rate of synthesis of the 180 day old rats being one-third that of the 22 day old. The rate of incorporation of label into sulfatide from $S^{35}O_4$ is lower in both rat liver and brain than S^{35}-Methionine. Rats fed a 5% casein diet had a significantly lower level of lipide sulfur and a higher rate of sulfatide synthesis in both liver and brain than animals fed a 25% casein diet. There appears to be no significant difference in sulfalipide concentration in brain and liver of different adult animals (rat, guinea pig, mouse, and rabbit). There appears to be no sex difference in animals of same age of lipide sulfur content of rat brain or liver. (U.S. Atomic Energy AT(11-1)-479)

8 *Biochemistry*

THE STUDY OF SERUM LIPIDS BY DIRECT PAPER CHROMATOGRAPHY. G. V. Marinetti and C. Waterhouse.* University of Rochester School of Medicine and Dentistry, Rochester, N. Y.

Human serum (or plasma) can be chromatographed directly on silicic acid impregnated paper. The need for prior extraction of the lipids by solvents is eliminated. The phosphatides are separated with diisobutyl ketone-acetic acid-water 40:20:3 and the nonphosphatides with n-heptane-diisobutyl ketone 96:6. Chromatography is carried out on sheets of paper 20 x 21 cm. in 2 qt. large mouth Mason jars at 23°C. 10 to 20 μl of serum are used. Pancreatin acts immediately on serum lipoproteins to hydrolyze triglycerides to diglycerides and fatty acids. After 1 to 3 hours the diglycerides are resynthesized to triglycerides with a concomitant disappearance of fatty acids. Phospholipase A acts immediately on serum lipoproteins to hydrolyze lecithin to lysolecithin and fatty acid. Versene completely inhibits the action of phospholipase A but has no detectable effect on pancreatin. Calcium ions overcome the versene inhibition. Analysis of many human serum samples has shown that triglycerides are the most variable component. Lipomul was found to contain triglycerides, diglycerides, fatty acids, lecithin, and lysolecithin. Intravenous infusion of Lipomul into patients demonstrates that all these lipids are rapidly metabolized. Direct chromatography facilitates the study of all the major serum lipids.

9 *Biochemistry*

LIBERATION AND STIMULATION OF ADIPOSE TISSUE LIPASES. Louise Mashburn*, Rose Brown*, and W. S. Lynn. Duke Univ. Medical Center, Durham, N. C.

Previous reports have indicated that epinephrine and ACTH promote the liberation of fatty acids and glycerol from adipose tissue. By using naphthol acetate, naphthol caprylate and glycerol butyrates, three different esterases can be demonstrated to be present in adipose tissue. Naphthol acetate esterase (I) is released from adipose tissue into the incubating medium in the presence of iodoacetate (10^{-4}M) or hypotonic buffer. Other metabolic inhibitors also cause release of (I) to a lesser degree, but they do not cause the release of tributyrinase (III) or naphthol caprylate esterase (II). (II) is released in the presence of isotonic or hypertonic buffer. Tissue homogenates, however, invariably hydrolyze naphthol acetate at least four times as actively as naphthol caprylate. (III) is liberated from the tissue only upon homogenization. Epinephrine and ACTH stimulate the hydrolysis of tributyrin, but not of the naphthol esters, by adipose tissue in vitro. No stimulation of (III) by these two hormones on tissue homogenates has been observed. It can be concluded, therefore, that adipose tissue contains at least three types of esterases, only one of which is effected by epinephrine and ACTH. Further characterization and separation of these three activities is now in progress.

10 *Biochemistry*

VINYLACETYL ISOMERASE. H. C. Rilling* and M. J. Coon. Dept. of Biological Chemistry, University of Michigan, Ann Arbor.

The unexpected observation was made earlier [Biochim. et Biophys. Acta, 21, 290 (1959)] that β-methylvinylacetyl CoA (MVA CoA) can apparently substitute for β-methylcrotonyl CoA (MC CoA) in a system containing MC CoA carboxylase (purified from chicken liver and freed of enoyl hydrase). Similar results have been obtained with MC CoA carboxylase partially purified from a Pseudomonas isolated in this laboratory from soil by enrichment culture technique. An isomerase which converts MVA CoA to MC CoA has recently been found in liver extracts. The enzyme, free of enoyl hydrase, is assayed spectrophotometrically by measuring the rate of increase in absorbancy at 266 mμ, due to the formation of MC CoA from synthetically prepared MVA CoA. The isomerase partially purified from ox liver is also active with vinylacetyl CoA and certain other similar compounds, and requires no cofactors. The isomerase has a pH optimum of 7.8 and is sensitive to sulfhydryl-binding agents. In the presence of the purified bacterial carboxylase, MVA CoA is not active unless the purified isomerase is added. The possible role of the isomerase in fatty acid metabolism is being studied.

11 *Biochemistry*

HORMONE EFFECTS ON GLUCOSE UPTAKE AND PHOSPHORYLASE ACTIVITY IN ADIPOSE TISSUE. M. Vaughan. Natl. Heart Inst., NIH, Bethesda, Md.

Epinephrine, norepinephrine, ACTH and glucagon all stimulate the release of free fatty acids (FFA) when present in the medium during in vitro incubation of rat adipose tissue. Employing paired epididymal fat bodies from single rats (one incubated with, the other without additions) we have determined the effects of these hormones on FFA release, glucose uptake and tissue phosphorylase activity. Tissues incubated with each of these 4 hormones exhibit, in addition to the expected increase in FFA release, increased rates of glucose uptake and levels of phosphorylase activity significantly greater than those of the paired control tissues at the end of 1 hour. There appear to be some quantitative differences in the relative effects of the 4 hormones on these 3 parameters and also on the conversion of U-C^{14}-glucose to triglyceride glycerol and fatty acids. Other workers have shown that the effects of these 4 hormones in other susceptible tissues are mediated through the formation of cyclic-3',5' adenosine monophosphoric acid. No effect of this nucleotide on glucose uptake or on phosphorylase activity is demonstrable in our studies although FFA release is decreased. The mechanisms of these hormone effects are under continued study.

12 *Biochemistry*

SULFANILAMIDE ACETYLATION WITH ACETATE AND ETHANOL. Robert Snyder*, Martin P. Schulman, and W. W. Westerfeld. Dept. of Biochem., State U. of N. Y. Med. Coll., Syracuse.

The acetylating system in 40% pigeon liver homogenates (Lipmann, J. Biol. Chem. 160, 173, 1945) was studied with C-14 acetate and ethanol by determining C-14 in acetylsulfanilamide (AcSulfa) and the total amount of AcSulfa colorimetrically. The incubation time was 40 min. and the final volume was 1.5 ml. A maximum stimulation of acetylation was produced by 4 μmoles of added acetate; the stimulation varied inversely with the rate of endogenous acetylation. Using C-14 acetate, the isolated AcSulfa contained more C-14 than predicted from the amount of AcSulfa formed in excess of the endogenous levels. Either the added acetate replaced part of the endogenous acetyl donor or it "exchanged" with AcSulfa. When unlabeled AcSulfa (200%) was incubated with homogenate containing C-14 acetate, the reisolated AcSulfa accounted for only 1/10 of the excess C-14 incorporation. 4 μmoles of ethanol stimulated acetylation only slightly, and the AcSulfa contained the predicted amount of C-14. 20 μmoles of ethanol did not increase total acetylation, but gave appreciable C-14 in AcSulfa. It was not possible to detect free acetate formation during ethanol metabolism either by dilution experiments or by isolation of C-14 acetate after incubation. (Aided by PHS M-1947.)

13 *Pharmacology*

TISSUE DISTRIBUTION OF C-14 AFTER THE INTRAVENOUS INJECTION OF LABELED FREE FATTY ACIDS (FFA) IN THE NEPHROTIC RAT. C. L. Malmendier (intr. by J. H. Baxter.) Natl. Heart Inst., NIH, Bethesda, Md.

The tissue distribution of C^{14}-FFA in normal rats has been studied by Bragdon and Gordon (J.Clin.Invest. 37:574, 1958). The present study was made to determine whether there are abnormalities in distribution in nephrotic rats (fasting or carbohydrate-fed), which might point to the origin of the hyperlipemia. The percentage of C^{14} in lipid extracts of liver, 10 or 200 minutes after injection, was significantly greater in nephrotic animals whether fasting or carbohydrate-fed; the percentage in muscle was changed in inverse proportion to that of liver. The percentage of radioactivity remaining in the blood was 3 to 4 times greater than in normal rats; this increase resulted primarily from an abrupt rise in labeling of neutral lipids, the label in the FFA being only slightly more than in normal rats. A correlation existed between the degree of these changes and the degree of hyperlipemia, the distribution in proteinuric rats without hyperlipemia being similar to that in normal rats. These differences seem most likely to be due to an increase in synthesis and delivery to plasma of lipoprotein lipids by the liver. These disturbances in liver metabolism possibly are induced by concentrations of plasma albumin below a critical level.

14 *Pathology*

ON THE MECHANISM OF HYPERLIPEMIA IN EXPERIMENTAL NEPHROSIS. Richard E. Tracy,* Randolph H. Hughes,* and Robert W. Wissler. Department of Pathology, University of Chicago, Chicago, Ill.

Studies have been undertaken to explore the mechanism of lipemia which occurs in rats after the administration of anti-kidney serum prepared by immunizing rabbits to rat kidney. The degree of lipemia has been found to correlate equally well with the extent of urinary loss of all protein fractions as determined by paper electrophoresis of urine. Results previously reported (Fed. Proc. 18(1):2014) demonstrated the lipid lowering effect of the subcutaneous administration of protein from nephrotic rat urine. Further studies have now revealed that this lipid lowering effect can be duplicated by similar administration of normal rat urine. Evidence will be presented to support the concept that the reduction in serum lipids produced by these substances may be mediated by a primary action of decreasing urinary albumin output. Supported by a grant from the United States Public Health Service #2G-93, Medical Training.

15 *Nutrition*

LIPID METABOLISM OF YOUNG MEN IN ALABAMA. Frieda L. Meyer and Doris D. Taylor*, School of Home Economics, University of Alabama, Tuscaloosa, Alabama, and Human Nutrition Research Division, USDA, Washington, D. C.

Seven young men served as subjects during a 50-day metabolism study using a controlled diet. The linoleic acid content of the dietary fat was 10% during the first 25 days and 25% during the second 25 days. Fat supplied 40% of the calories for all subjects with food energy intake adjusted for individual caloric requirement. The cholesterol levels for three men were higher at the end of the 10% linoleic acid period than the levels immediately preceding the controlled diet period. After 10 days on 25%, all seven men showed some lowering in blood cholesterol, but at the end of 25 days two had returned to their levels on the 10% linoleic acid period. Cholesterol values for six subjects were higher after 60 days on self-selected diets than at the end of the controlled diet period. At the end of 50 days on the controlled diet the variation among six of the men was considerably reduced from that found for the men on their self-selected diets. (Supported in part by a USDA contract sponsored by the Human Nutrition Research Division, Agricultural Research Service.)

16 *Nutrition*

LIPID METABOLISM OF YOUNG MEN IN WASHINGTON STATE. Mary R. Gram*, E. A. Donald* and Hilda F. Wiese, College of Home Economics, Washington State University, Pullman, Washington, and Department of Pediatrics, University of Texas School of Medicine, Galveston, Texas.

Six healthy men, 22 to 26 years of age, received a controlled mixed diet with 40% of the calories from fat. The fat, a blend of the kinds and proportions typical of average U. S. consumption, contained 10% linoleic acid the first 20 days. Modifications were made to give 20 and 30% of linoleic acid for 2 periods of 15 days each. Four of the men had lower serum cholesterol on the 30 than on the 10% linoleic acid. The average serum cholesterol and total fatty acids for the 6 subjects did not change significantly as dietary linoleic acid increased. The percentage of dienoic acid in the phospholipid, cholesterol ester and glyceride fatty acids tended to increase progressively in the serum as dietary linoleic acid increased. The amount of fat excreted in the feces, expressed as percent of intake, remained unchanged. There was considerable variation in the retention of nitrogen, calcium, phosphorus and magnesium. (Supported in part by a USDA contract sponsored by the Human Nutrition Research Division, Agricultural Research Service.)

17 *Nutrition*

LIPID METABOLISM OF YOUNG MEN IN ARKANSAS. M. Isabel Irwin* and Hilda F. Wiese. Dept. of Home Economics, University of Arkansas, Fayetteville, Arkansas, and Dept. of Pediatrics, University of Texas School of Medicine, Galveston, Texas.

Six men were fed a controlled diet containing blends of fat with varying amounts of linoleic acid (10, 20, and 30%) in successive periods. The fat blend with 10% linoleic acid is representative of average fat consumed in the USA. The diet provided about 40% of calories from fat. Serums from venous blood drawn at beginning of study and end of each period were analyzed for total cholesterol and for fatty acids in the different lipid fractions. Food and excreta were analyzed for fat, nitrogen, phosphorus, calcium, and magnesium. Five of 6 men had higher serum cholesterol levels after the first period (10% linoleic acid) than at the beginning indicating importance of previous diets among other factors. At the close of the period on 30% linoleic acid the total serum cholesterol for the 6 men ranged from 15 to 51 mg % lower than on the 10% level. There were significant changes in lipid composition of fractions but not in amount of total serum fatty acids. Most obvious change was an increase in dienoic acids, especially in tri-glyceride fraction. The biological significance of changes in retention of nitrogen and minerals is discussed. (Supported in part by USDA contract sponsored by the Human Nutrition Research Division, Agricultural Research Service.)

147. LIPIDS—Fats and Fatty Acids—Synthesis

1 *Physiology*

AN ANALYSIS OF THE DEPRESSED HEPATIC LIPOGENESIS INDUCED BY COLD STRESS. E.J. Masoro, Dept. of Physiology, Tufts Univ. School of Medicine, Boston, Mass.

Liver homogenates for lipogenic studies were prepared from fed rats living at 25°C (control rats), fed rats exposed to 0-2°C for 1 day (cold-fed rats) and fasted rats exposed to 0-2°C (cold-fasted rats). Liver homogenates from cold-fed rats synthesized fatty acids about as well as did homogenates from control rats. The conclusion is drawn that the failure of lipogenesis in the intact liver cell of the cold-fed rats does not result from a lack of fatty acid synthesizing enzymes but is the result of an unfavorable co-factor environment. Evidence is presented indicating that lipogenesis fails in the intact liver cell of the cold-fed rat because of a depressed TPNH generation by way of the hexose monophosphate pathway of carbohydrate metabolism. Liver homogenates from cold-fasted rats were unable to synthesize fatty acids even in a co-factor environment found favorable for lipogenesis in homogenates from cold-fed and control rats. This failure in lipogenesis is not due to a lack of TPNH generation or of carbohydrate metabolism over either the hexose monophosphate pathway or the Embden-Meyerhof pathway. It is concluded that either the fatty acid synthesizing enzymes are almost absent or a necessary unknown co-factor is not present at adequate levels in the liver of the cold-fasted rat.

XUM

2 *Physiology*

REGULATION OF FATTY ACID SYNTHESIS AND DIRECT OXIDATIVE PATHWAY IN ADIPOSE TISSUE. B. Leboeuf and G. F. Cahill, Jr. (intr. by G. W. Thorn), Harvard Medical School, Boston, Mass.

Insulin added in vitro to adipose tissue from normal fed rats accelerates glucose metabolism by both direct oxidative (DOP) and Embden-Meyerhof (EMP) pathways. Relative recoveries of glucose carbon-6 in fatty acids (FA) and CO_2 permit estimation of two alternative pathways of acetyl-CoA metabolism in this tissue, namely fatty acid synthesis and oxidation by the TCA cycle. The CO_2/FA ratio in control tissues is 0.8 to 1.2 and with insulin decreases to 0.15. The increased glucose uptake caused by epinephrine, or by ACTH or growth hormone preparations is not associated with the expected increase in fatty acid synthesis and CO_2/FA ratios are 17, 22 and 20 respectively. Calculations of the contribution of DOP to total glycolysis (40% in controls) are 7, 18 and 13% after addition of these hormones. Similar changes are produced by 2,4-dinitrophenol (10^{-4}M), namely increased glucose uptake, proportionately less DOP and increased CO_2/FA ratio. Although current opinion favors the control of fatty acid synthesis by TPNH produced by DOP, these data suggest the alternative possibility that acetyl-CoA availability for fatty acid synthesis regulates DOP.

3 *Physiology*

THE "REPAIR" OF LIPOGENESIS ON RE-FEEDING: THE EARLY HOURS. Jay Tepperman, Helen M. Tepperman and Janet DeWitt*. Dept. of Pharmacology, SUNY, Upstate Medical Center, Syracuse, N. Y.

Examination of liver slices of rats refed a high carbohydrate diet following a 48 hr. period of starvation revealed striking increases in the amount of carbon 1 of glucose that appeared in respiratory CO_2. (The Authors, Fed. Proc. 18: 157, 1959). The shift in C-6/C-1 ratios at 24 and 48 hrs. post re-feeding paralleled the markedly increased lipogenesis rates. The present study was done to secure an answer to the question: does the shift in C-6/C-1 ratio anticipate the recovery of lipogenesis from acetate-1-C^{14}? Livers of rats (6-8 per group) were studied 30, 60, 90, 120 and 240 minutes after the animals had been fed a complete, high carbohydrate diet by stomach tube. Liver glycogen, lipogenesis from acetate-1-C^{14} and CO_2 formation from glucose-1-C^{14} and glucose-6-C^{14} were measured. At 60 minutes no recovery of lipogenesis was apparent, although glycogen deposition had begun at 30 minutes. By 240 minutes lipogenesis rate was significantly above that of the starvation control levels ($P < 0.01$). During most of the 120 minute period, however, there was a highly significant comparative decrease in CO_2 from glucose-1-C^{14} with a consequent shift of the C-6/C-1 ratio in the opposite direction from that seen after 24 and 48 hrs. of re-feeding. (Supported by a USPHS grant.)

4 *Biochemistry*

THE CONDENSATION OF MALONYL COA WITH ACETYL OR BUTYRYL COA. John W. Porter, Edward A. Steberl* and Gertrude W. Wasson*. WA Hospital and Department of Physiological Chemistry, University of Wisconsin, Madison, Wisconsin.

Previous reports (Wakil and Ganguly, J. Am. Chem. Soc. 81: 2597, 1959; Long and Porter, J. Biol. Chem. 234: 1406, 1959) have shown that malonyl and butyryl CoA are intermediates in the biosynthesis of palmitic acid by a soluble pigeon liver enzyme system. Further fractionation of this system has yielded an enzyme fraction that condenses malonyl CoA with either acetyl or butyryl CoA. The condensation product (R_f 0.25 - 0.30) is separated from each of the parent compounds by paper chromatography in an ammonia-isobutyric acid system. A condensation product of malonyl CoA and butyryl CoA is also formed by carrot root plastids. The condensation product may be converted to the hydroxamate derivative, and separation from the hydroxamates of acetate, malonate and butyrate is achieved on paper chromatography in a water-saturated butanol system. The condensation product is reduced with one equivalence of TPNH by another partially purified enzyme obtained from pigeon liver. The R_f value of the reduction product is only slightly changed from that of the condensation product. This fact is evidence that the carboxyl group of malonyl CoA is still retained in the first reduction product.

5 *Biochemistry*

INCORPORATION OF GLYCEROL INTO LIPIDES OF BULL SPERMATOZOA Charles Terner and Gregory Korah*. Worcester Fdn. for Exptl. Biology, Shrewsbury, Mass. and Dept. of Biology, Boston Univ., Boston, Mass.

Washed bull spermatozoa were incubated aerobically with acetate-1-C^{14} and glycerol-1-C^{14} at the same time. The lipids were extracted and fractionated by silicic acid and paper chromatography. The fraction of highest specific activity ran with cholesterol which, however, when isolated as the digitonide was found to be unlabeled. The remaining radioactive fraction was freed from digitonin and crystallized from MeOH; (m.p. 60-61°). Ester groups were indicated by the alkaline hydroxylamine test. Infra red spectra showed -CO, -OH and the progression bands characteristic of long-chain fatty acids. The chromatographic behavior on silicic acid and on paper suggested the material to be mainly diglycerides. On saponification, the fatty acid fraction was found to be unlabeled, whereas the glycerol (isolated by paper chromatography) was strongly radioactive. Aerobic incubation of spermatozoa with glycerol-1-C^{14} alone and aerobic or anaerobic incubation with glucose-C^{14} (r.l.) resulted in the appearance of the same labeled material. From the phosphatide fractions which were also labeled a radioactive component, containing choline, was isolated and crystallized; its infra red spectrum corresponded to that of lecithin. Supported by Grant RG-4963, National Institutes of Health

6 *Biochemistry*

DECARBOXYLATION OF MALONYL CoA DEPENDENT UPON THIOLESTERS OF CoA. P.R. Vagelos and A.W. Alberts (intr. by B.N. Ames). Natl. Heart Inst., Bethesda Md.

Enzyme preparations of Clostridium kluyveri catalyze an exchange reaction between malonyl CoA and $HC^{14}O_3^-$ that is dependent upon the presence of straight chain, unsubstituted thiolesters of CoA (Vagelos, J.Am.Chem.Soc.81, 4119, 1959). All the CoA esters tested except acetyl CoA cause a comparable stimulation of this exchange reaction. Acetyl CoA stimulates only slightly. Succinyl CoA will not replace malonyl CoA. The exchange is inhibited by p-chloromercuribenzoate and arsenite but not by avidin. When 3-C^{14}-malonyl CoA was incubated with this enzyme in the absence of HCO_3^-, the liberation of $C^{14}O_2$ was dependent upon the addition of the same thiolesters as listed above. The other product(s) of this decarboxylation reaction have not yet been identified. When malonyl CoA was incubated with 1-C^{14}-caproyl CoA and enzyme, no new radioactive product could be detected. However, β-ketooctanoyl CoA, a possible decarboxylated product of the reaction between malonyl CoA and caproyl CoA, was rapidly cleaved to acetyl CoA and caproyl CoA when it was incubated with this enzyme.

7 *Biochemistry*

SYNTHESIS OF FATTY ACIDS BY PARTICULATE PREPARATIONS FROM AVOCADO MESOCARP. J.B.Mudd** and P.K.Stumpf. Department of Biochemistry, University of California, Davis, California.

Particulate preparations from avocado mesocarp incorporated acetate-1-C^{14} into lipid. Label was found mainly in triglyceride, but also in phospholipid and free fatty acids. Methyl esters of the fatty acids from these groups have been separated gas chromatographically, trapped and assayed for radioactivity. The esters collected for counting were of: acids less than C_{16}; palmitic and palmitoleic (C_{16}); "C_{17}"; stearic; oleic; and linoleic. All fractions became labelled to some extent. In time course experiments, the earliest samples showed the greatest proportion of the label in acids less than C_{16} (37% after 10 minutes). However, the C_{16} fraction rapidly became predominant (46% after 25 minutes). The proportions of the fractions became constant as the time of exposure to acetate increased. (After 100 minutes < C_{16}, 4%; C_{16}, 41%; "C_{17}", 3%; stearate, 32%; oleate, 16%; linoleate, 5%.) Stearate, which occurs in very small amounts in the natural lipid, forms a significant proportion of the lipid synthesized in vitro, and oleate, the predominant fatty acid in the natural lipid, is seldom synthesized in large proportions (up to 60% but usually 10-20%). (*Fellow of the Jane Coffin Childs Memorial Fund for Medical Research)

8 **Biochemistry**

TRIGLYCERIDE SYNTHESIS AND FREE FATTY ACID (FFA) RELEASE IN ADIPOSE TISSUE HOMOGENATES. D. Steinberg, M. Vaughan, S. Margolis* and A. Karmen*. Natl. Heart Inst., NIH, Bethesda, Md.

Adipose tissue, being in a dynamic state, the release of FFA from it may be importantly influenced by factors altering the rate of reesterification of FFA into triglyceride (TG). The mechanism of TG synthesis was explored in homogenates of rat epididymal fat pad. Optimal incorporation of 1-C^{14}-palmitate was obtained on adding: α-glycerophosphate, ATP, CoA, Mg^{++}, F^- and cysteine. α-glycerophosphate could be replaced by dihydroxyacetone-P, fructose-1,6,-diphosphate or glucose-6-phosphate but not by glycerol. 50 to 80% of incorporated radioactivity was found in TG and most of the remainder in diglycerides. Specific radioactivity of the latter was about 100 times that of the former. Only traces of radioactivity were found in cholesterol esters and less than 5% in phospholipids (including phosphatidic acid). Results are compatible with synthesis by a mechanism similar to that described for TG synthesis in liver (Weiss and Kennedy, J.A.C.S. 78, 3550(1956)). Relative rates of incorporation into various TG fractions will be reported.

In unfortified homogenates there was net release of FFA; with the full system there was generally net uptake of FFA. The relation of these studies to hormonal effects on FFA release in adipose tissue will be discussed.

9 **Biochemistry**

A PRIMARY DEFECT IN FATTY ACID BIOSYNTHESIS IN LIVER OF THE ALLOXAN-DIABETIC RAT.
David M. Gibson, Dorothy D. Hubbard*, and W. Clayton Love*. Dept. Biochem., Indiana Univ. School of Med., Indianapolis, Ind.

The fatty acid synthesizing system (Gibson, et al., Biochim. Biophys. Acta, 30 (1958) 376) that (a) is prepared from liver supernatant, (b) requires CO_2 (c) requires TPNH and (d) is inhibited by avidin has been studied in soluble preparations from liver of alloxan-diabetic rats. The unfractionated supernatant and the derived dialyzed subfractions that are prepared from diabetic liver are incapable of fatty acid biosynthesis although these systems either contain or are supplimented with a TPNH-generating system (isocitric dehydrogenase). Treatment of alloxinized rats with insulin or by adrenalectomy restores the biosynthetic activity to normal levels in the liver enzyme system subsequently isolated. Thus a primary defect in this fatty acid synthesizing system obtains in liver from the alloxan-diabetic rat that cannot be explained in terms of TPNH generation.

10 **Biochemistry**

PROPIONATE INCORPORATION INTO A UNIQUE BRANCHED FATTY ACID. J. W. Corcoran*, T. Kaneda*, J. C. Butte* (intro. by H. Sable) Dept. of Biochem., Western Reserve U., Cleveland, Ohio.

The antibiotic erythromycin contains a unique branched chain lactone ring called erythronolide (I), made up entirely of repeating 3 carbon units (--$CH(CH_3)\cdot CH_2$--). Propionate has been proposed as the precursor of the 3 carbon unit. Washed resting cells of S. erythreus incorporate the carbon of propionate-1-, or -2-C^{14} into (I) about 60 and 35 times more effectively than succinate-1, 4- and -2, 3-C^{14} respectively and 600 and 250 times more than acetate-1- or -2-C^{14} respectively. Chemical degradation of (I) shows C-1 of the propionate is incorporated only into methylene carbons of (I), whereas C-2 is incorporated largely into the tertiary carbon atoms with no incorporation into the carbon bound methyl groups. The data suggest that the precursor of (I) is closely related to propionate. Vanek et al. (AEC Int. Cong., Geneva, 1958) have found that propionate is an efficient precursor of (I), but degradation of (I) was not reported.

11 **Biochemistry**

Biosynthesis of Lactobacillic Acid. Teh-yung Liu* and Klaus Hofmann. Biochemistry Department, University of Pittsburgh School of Medicine, Pittsburgh, Pa.

Unequivocal evidence is presented in support of the concept (Hofmann et al., J. Biol. Chem., 228, 349 (1957) that the cyclopropane ring of lactobacillic acid arises from addition of a one-carbon fragment across the double bond of cis-vaccenic acid. Lactobacillic acid isolated from lactobacillus arabinosus cells grown on either formate-C^{14} or methionine-methyl-C^{14} contained significant amounts of isotope.

Degradation of the labelled lactobacillic acid according to a procedure developed in this laboratory (Hofmann et al., J. Am. Chem. Soc., 80, 5717 (1958) gave iodoform-C^{14} containing more than 80 per cent of the original radioactivity. Since this iodoform is derived exclusively from the methylene-bridge carbon the radioactivity of the lactobacillic acid must have resided in this position.

In conjunction with our previous demonstration (Hofmann and Liu, Biochim. Biophys. Acta, in press) of the direct utilization of cis-vaccenic acid in lactobacillic acid biosynthesis by L. arabinosus these findings establish the source of the entire carbon skeleton of lactobacillic acid.

12 **Biochemistry**

EFFECT OF PSEUDOVITAMIN B_{12} ON THE ENZYMATIC CARBOXYLATION OF ACETYL COENZYME A. Roscoe O. Brady and Joseph V. Formica*. National Institutes of Health, Bethesda, Maryland.

Investigations of the carboxylation of acetyl CoA (Formica, J. V., and Brady, R. O., J. Am. Chem. Soc. 81, 752 (1959)) have been extended with a partially purified preparation from the high speed supernatant solution of extracts of rat liver tissue obtained by fractional precipitation between 20 and 30% saturation with $(NH_4)_2SO_4$. The carboxylation of acetyl CoA was 50 % inhibited by 0.01 M KCN. The addition of 5×10^{-7} M pseudovitamin B_{12} caused a 70% stimulation of the amount of acetyl CoA converted to malonyl CoA. The presence of the same quantity of B_{12} or dimethylbenzimidazole cobamide coenzyme effected 26% increase over the control values. Vitamin B_{12} minus the benzimidazole riboside was without effect. In the absence of acetyl CoA, B_{12} and its derivatives at these concentrations do not act as substrates for the fixation of $C^{14}O_2$. Propionyl CoA carboxylase was most active in the 0-20% $(NH_4)_2SO_4$ fraction and was unaffected by the addition of KCN or pseudovitamin B_{12}.

13 **Biochemistry**

BIOSYNTHESIS OF FATTY ACIDS. Salih J. Wakil. Department of Biochemistry, Duke Univ. Med. Center, Durham, N. C.

The synthesis of long-chain fatty acids (palmitate) from acetyl CoA in the presence of ATP, Mn++, HCO_3 and TPNH, is catalyzed by two enzyme fractions (R_{1gc} and R_{2gc}) derived from avian livers. Monomalonyl CoA was demonstrated to be the first intermediate in this series of reactions and its synthesis is catalyzed by the biotin-containing enzyme fraction R_{1gc}. Further evidence shows that monomalonyl CoA can be readily converted to long-chain fatty acids in the presence of the enzyme fraction (R_{2gc}) and TPNH. C14-acyl CoA, (acetyl CoA, butyryl CoA, and octanoyl CoA) can be incorporated into palmitate in the presence of the forementioned system. These observations led us to propose a series of reactinns involving the intermediate formation of dicarboxylic acyl CoA, [Wakil, S.J. and Ganguly, J.E., J. Am. Chem. Soc., 81, 2579, (1959)] . On further purification of R_{2gc}, two different subfractions are obtained which on recombination reconstitute the original system. In the reconstituted system the rate of oxidation of TPNH in the presence of malonyl CoA is markedly increased by the addition of acetyl CoA. The subfractionation and purification of R_{2gc} will be described.

XUM

14 *Biochemistry*

CHLORPROMAZINE AND C^{14} ACETATE INCORPORATION INTO FATTY ACIDS AND CHOLESTEROL. J.A. Christensen* and A.W. Wase* (intr. by M.J. Boyd). Hahnemann Medical College, Philadelphia, Pa.

Clinical observations indicate prolonged treatment with chlorpromazine (CPZ) and related compounds leads to weight gains due largely to accumulated adipose tissue. The present studies from these labs. indicate CPZ to increase the incorporation of C^{14} from 1-C^{14} acetate into total fatty acids, esp. in liver. Male rats, grouped for equal wt. received various doses of CPZ and 40 uc of C^{14} acetate. Rats were killed at various times, livers and brains removed and fatty acids and chloresterol isolated by conventional methods following hydrolysis of the total lipids extracted by $CHCl_3$-MeOH (2:1). Specific activities of the isolated materials were determined by standard counting procedures. In brain, the specific activity of the fatty acids was decreased 10 & 8% after 3 hrs. at 10 & 20 mg/Kg CPZ respectively showing a slight increase of 6% at the 40 mg/Kg dose level. Liver fatty acids were decreased 23-25% at the 5 & 10 mg/Kg dose level and increased 28% at the 30 mg/Kg level. Non-fasted rats showed increases over 300% at the 20 & 30 mg/Kg dose levels of CPZ. Mellaril, a similar drug, gave nearly identical responses. Brain cholesterol exhibited no remarkable responses, but liver cholesterol specific activities were increased 200 & 220 % at 2 & 3 hrs. following a 40 mg/Kg dose of CPZ. Increases of the order of 200 % were also found in the specific activities of cholesterol isolated after 3 hrs. from livers of rats receiving 10,20 & 30 mg/Kg CPZ. From these studies it appears that high doses of CPZ increases the synthesis of fatty acids and cholesterol in liver. (Supported by USPHS-NIH Grant M1383(C2)).

15 *Pharmacology*

BUTYRIC ACID INCORPORATION IN BOVINE MILK FAT SYNTHESIS. Soma Kumar and Karl Lalka (intr. by V.S. Waravdekar.) Georgetown University, Washington, D.C.

The location of the butyric acid in bovine milk fat was determined by taking advantage of the specificity of pancreatic lipase for the fatty acids at the α -carbon atoms of glycerol. 2 gm of milk fat, emulsified with bile salts in 360 ml Tris buffer (pH 7.0), was incubated for 3 minutes with commercial pancreatic lipase at 39°C. The reaction was stopped by adjusting the pH to 2.0 and the sample extracted with alcohol-ether mixture; the extract was then washed free of fatty acids with dilute Na_2CO_3 and the remaining neutral glycerides were isolated. The butyric acid content in this was 2.4 molar % which represented a 76.5% reduction from that of the butter fat used. An appreciable exchange of free volatile fatty acids was found to occur in this reaction; hence the butyric acid remaining in the glycerides, most probably, represents the fraction present under equilibrium conditions. At any rate, a large fraction, if not all, of the butyric acid of the milk triglycerides is located in the α-positions. The data suggest that the butyric acid synthesized in the lactating ruminant mammary gland is incorporated into the milk triglycerides by condensation with 1,2-diglyceride, presumably formed from the blood neutral fats. (Supported by the U.S. Atomic Energy Commission Grant No. AEC (30-1)-2412.)

148. LIPIDS—Fats and Fatty Acids—Transport

1 *Physiology*

THE EFFECT OF SYMPATHETIC BLOCKADE ON PLASMA FREE FATTY ACIDS. William T. McElroy, Jr.* and John J. Spitzer. Hahnemann Medical College and Hospital, Philadelphia, Pa.

The sympathetic nervous system is thought to have an important regulatory effect on the release of free fatty acids (FFA) from adipose tissue. The present report deals with the effects of sympathetic blockade at the ganglionic or postganglionic levels on FFA content of the blood. Dogs were given intravenously Arfonad (Trimethaphan), Dibenzylene, or TM-10 (choline-2:6-xylyl ether bromide). Arfonad increased plasma FFA levels, and venoarterial FFA differences in the leg. Simultaneous hepatic plasma flows (as determined by the BSP dilution method) were reduced with inconsistent changes in the uptake of FFA by the liver. Dibenzylene had a similar effect on FFA levels and venoarterial differences as Arfonad, and increased FFA uptake by the liver. TM-10 also raised plasma FFA levels. Arfonad, or Dibenzylene failed to block the metabolic response of FFA and blood sugar to the inhalation of high CO_2 mixtures, a stress known to induce sympathetico-adrenal discharge. (Supported by the National Institutes of Health.)

2 *Physiology*

DISAPPEARANCE CURVES OF I.V. ADMINISTERED I^{131} LABELLED TRIOLEIN IN HUMANS. Leonard J. Feinberg, Herschel Sandberg, Edward R. Dickstein, and Samuel Bellet (Introduced by Raymond Penneys) Div. of Cardiology, Phila. Gen Hosp., Phila.

Previous studies indicate a difference between patients with myocardial infarction (M.I.) and controls in blood levels of I^{131}, both whole blood (W.B.) and TCA precipitable lipoprotein fraction (TCA), following an oral I^{131} labelled Triolein test meal. In the present study the influence of G.I. absorption is by-passed by I.V. administration of I^{131} labelled Triolein emulsion to 9 controls and 5 M.I. patients. No significant difference between these two groups was found. Typical disappearance curves show: 1. W.B. I^{131} disappears rapidly the first five min. ($T\frac{1}{2}$ = 2 to 4.5 min.), less rapidly the next 5-7 min. reaching a minimum at 15 min. It then rises for a variable interval reaching a new maximum at approx. 60 min. and then slowly falls again. 2. The TCA curve parallels the W.B. the first 5-7 min. then falls more rapidly; the rise starts later and is of shorter duration. 3. The TCA/WB% curve drops very slowly the first 5-6 min. and then rapidly till the 20th min., then levels to the 60th min., plateauing at about 2 hours. The curves agree with those found in dogs using I^{131} labelled chylomicra (Am. J. Physiol. 193:294'58) and in normal humans using albumin bound C^{14} palmitate (J.C.I. 37:1504'58).

3 *Physiology*

CHANGES IN THE COMPOSITION OF PLASMA NON-ESTERIFIED FATTY ACIDS AFTER INSULIN, GLUCOSE, AND NOR-EPINEPHRINE. Hans Meinertz* and Vincent P. Dole. Rockefeller Institute, New York.

Arachidonic acid (20:4) normally accounts for about 7% of the non-esterified fatty acid (NEFA) fraction in rat plasma. It is, however, virtually absent from adipose tissue, which appears to be the major source of NEFA. Since the lowering of NEFA in response to insulin or glucose, and the rise after epinephrine, can be attributed to changes in the outflow of fatty acids from adipose tissue, it became of interest to fractionate the NEFA by means of gas-liquid chromatography and to examine the behavior of 20:4 during these responses. It was found that 20:4 remained essentially constant in absolute concentration; its percentage in the NEFA mixture varied inversely with the total NEFA concentration, while other fatty acids in the fraction tended to maintain constant relative proportions. The result suggests that 20:4 in NEFA originates elsewhere than adipose tissue and differs functionally from the other fatty acids in NEFA.

4 *Biochemistry*

IN VITRO ABSORPTION OF GLYCERIDE FATTY ACIDS. H. C. Tidwell and John M. Johnston.* Dept. of Biochem., Univ. of Tex., Southwestern Med. School, Dallas, Texas.

A study has been made to obtain additional information regarding the form in which glyceride fatty acids are absorbed. An in vitro preparation of small intestinal sacs from the rat was employed in an attempt to avoid changes which may occur in the lumen. Either FA-albumin complex, mono-, di-, or triglyceride emulsions (C^{14} labeled) were incubated on the mucosal side of the everted intestine. The activities in the mucosal and serosal solutions and intestinal tissue were then determined. The activity in each of these compartments was further fractionated into free fatty acids and individual glycerides. Only unesterified fatty acids appeared to have been readily absorbed and converted into triglycerides with this preparation. These results are comparable to those in the intact animal as evidenced by the conversion of fatty acids into triglycerides which appeared in chylomicron form on the serosal side. Di- and triglycerides were not appreciably absorbed. The slight absorption of monoglyceride could be accounted for by the 20 per cent hydrolysis produced by an intestinal enzyme which was found to be liberated into the mucosal solution and to be rather specific for monoglyceride hydrolysis. (Supported in part by grants from the Robert A. Welch Fndn. and the Natl. Insts. of Health, PHS.)

5 *Biochemistry*

THE RARE EARTH FATTY LIVER: PLASMA FREE FATTY ACIDS. Fred Snyder*, Nelson Stephens*, Lois A. Gerst*, and G. C. Kyker. Oak Ridge Institute of Nuclear Studies, Medical Division, Oak Ridge, Tennessee.

Cerium (2 mg/kg) and some of the other rare earths produce a fatty liver when injected intravenously into rats. The present report describes some changes which have been observed in the plasma of cerium-treated female rats (Carworth Farms-Nelson strain). Measurement of the plasma free fatty acids (FFA) according to the method described by Dole, shows that a marked increase in FFA occurs at 24, 30, and 48 hours after administration of the rare-earth element. This increase occurs in both fasted and ad libitum-fed rats injected with cerium. The total plasma protein is decreased in the cerium-treated rats, whereas the albumin fraction is elevated at 48 hours after the cerium injection. The data further suggest that at least part of the increased liver lipids caused by rare earths (atomic nos. 57 - 62) is from adipose tissue sites.

6 *Pharmacology*

IMPROVED MEDIA FOR AXENIC CULTIVATION OF CAENORHABDITIS BRIGGSAE (NEMATODA). Eder L. Hansen, Francis W. Sayre*, and E. A. Yarwood*. Kaiser Foundation Res. Inst., Richmond, Calif.

The chemically defined basal medium for C. briggsae has now been redesigned to reflect further the importance of nutritional balance and some of the accumulating information on intermediary metabolism. The new medium includes amino acids at levels proportional to their occurrence in the proteins of Escherichia coli, a satisfactory associate of C. briggsae in monoxenic cultures. Total amino acid concentration of the defined medium is 161 millimolar. Vitamins are included at five times the level of previously described media (Dougherty et al., Ann. N. Y. Acad. Sci. 77:179, 1959). The new formulation permits satisfactory serial subcultures to be maintained when supplemented in minute amounts with a variety of organic supplements. Current work is being directed toward isolation and characterization of the growth promoting factor(s) furnished by complex organic materials derived from such diverse sources as chick embryo, serum, liver, or bacteria. This work is being greatly facilitated by a new rapid screening technique in which nematodes, maintained in certain deficient media in which they survive but do not grow, respond immediately to introduction of preparations containing "growth factor(s)" by normal growth and reproduction. (Supported in part by N.I.H. Grant E-926.)

7 *Pharmacology*

EFFECT OF PROLONGED STRESS ON PLASMA UNESTERIFIED FATTY ACIDS. Samuel Mallov and Peter Witt. SUNY, Upstate Medical Center, Syracuse, N. Y.

To assess the effect of prolonged stress on plasma unesterified fatty acid (UFA) levels, rats were subjected to electric shocks or cold (2-3°C) for various intervals of time and plasma UFA then determined by the method of Dole (J. Clin. Invest. 35:150,1956). 250 gm. female Sprague Dawley rats were placed in cages on wire grids and subjected to shocks of 1 sec. duration each 2.5 or 1.25 min. Voltages were adjusted (20 to 60 v) for each rat to produce approximately the same (subjectively graded) response of jumping and squealing. Rats shocked for 1/2 hr. showed no increase in UFA concentrations; for 2 hrs., a mean increase of 32.2% over control values (0.468 vs 0.354 mEq/L); and for 4 hrs., a mean increase of 48.3% over control values (0.534 vs 0.360 mEq/L). All differences were significant. Fasted rats shocked for 2 hrs. showed the same effects as fed rats, but UFA levels in both shocked and control rats were higher than in the fed animals. Male Wistar strain rats placed in the cold without food for 1 hr. showed a mean increase of 23% in plasma UFA (0.450 vs 0.366 mEq/L); for 3 hrs., a mean increase of 26.9% (0.439 vs 0.346 mEq/L); and for 12 hrs., a mean increase of 85.0% (1.047 vs 0.566 mEq/L). Differences were significant. (Supported by a grant from the American Heart Association.)

8 *Pharmacology*

EFFECT OF ADRENERGIC BLOCKING AGENTS UPON CCl_4-INDUCED TRIGLYCERIDE DEPOSITION IN RAT LIVER. W.M. Butler, Jr.*, H.M. Maling, B. Highman and B.B. Brodie. Natl. Insts. of Health, Bethesda, Md.

We previously reported that adrenergic blocking agents inhibit triglyceride deposition induced in dog heart by catecholamines, and in rat liver by ethanol, CCl_4, and ethionine. This suggested that the deposition of fat by the latter compounds was mediated through catecholamines. The present study shows the relation between time of pretreatment with blocking agents and the extent of fat deposition. Injection of a large dose of a blocking agent (Dibenamine, phenoxybenzamine, or ergotamine) 2 to 8 hours before s.c. CCl_4 increased the triglyceride deposition in the liver; in contrast, the fat deposition and necrosis were decreased when a blocking agent was given 24 to 48 hours before the CCl_4. Smaller doses of Dibenamine elicited a decreased lipid deposition whether given 2 or 24 hours before s.c. CCl_4. These results and the fact that phenoxybenzamine blocks CCl_4-induced fat deposition in rat liver less completely than it blocks catecholamine-induced deposition in dog heart suggest that the effect of the blocking agents may be more complicated than we had pictured.

9 *Pharmacology*

TRIGLYCERIDE DEPOSITION IN RAT LIVER THROUGH DERANGEMENT OF FAT TRANSPORT BY VARIOUS CHEMICAL AGENTS. H.M. Maling, M.G. Horning*, W.M. Butler, Jr.*, B. Highman and B.B. Brodie. Natl. Insts. of Health, Bethesda, Md.

Triglyceride levels in rat liver were considerably elevated within 24 hours after administration of ethionine, ethanol, and CCl_4, but significant necrosis occurred only after CCl_4. Analysis by gas chromatography showed that the increased liver triglyceride had about the same percentage of linoleic acid as adipose triglyceride. This indicates that the compounds increased fat mobilization since linoleic acid is not appreciably synthesized by mammalian liver. Experiments in which the adipose triglycerides were labelled by pretreatment with acetate-C^{14} confirmed that transport to the liver had occurred. The triglyceride deposition was much greater in female than in male rats. Hypophysectomy completely prevented the triglyceride deposition induced by ethanol and partially the deposition by ethionine and CCl_4. Pretreatment with the adrenergic blocking agent, Dibenamine, reduced the triglyceride deposition induced by CCl_4 and ethionine in hypophysectomized rats and the deposition induced by all three compounds in normal rats.

10 *Pathology*

EFFECTS OF HEPARIN ON TRANSPORT OF INTRAVENOUSLY ADMINISTERED LIPIDS. E. Klein, S. Fine, W.F. Lever and D.D. Duckett (intr. by A. Cohen.) Tufts University School of Med., Boston, Mass.

Intravenous Fat Emulsions were administered to humans and to dogs. First neutral fat alone was administered; 3 days later the infusion was repeated in the same individual immediately following heparin injection. In the presence of heparin the blood levels of total fatty acids and of neutral fats and plasma turbidity were markedly lower and returned to normal ranges more rapidly than when lipids alone were administered; unesterified fatty acid levels rose to considerably higher levels but returned more rapidly to preinfusion levels. Lipolytic activity in plasma was slight or not demonstrable following fat infusion, whether or not preceded by heparin. On fractionation, however marked lipolytic activity was demonstrable when heparin was marked for one hour but had disappeared within two hours. Heparin appears to accelerate the removal of intravenously administered lipids from the blood stream and to increase the rate of hydrolysis of the neutral fats (Contract No. DA-49-007-MD-1011, U.S. Army Res. and Develop. Command, SGO.)

XUM

11 *Nutrition*

THE LABELING OF PHOSPHATIDIC ACIDS DURING THE ABSORPTION OF FATTY ACIDS. John M. Johnston and James H. Bearden. (Intr. by H. C. Tidwell) Univ. of Tex., Southwestern Med. School, Dallas.

The mechanism by which fatty acids are absorbed by the intestine is still unresolved. Previous reports from this laboratory have indicated that this process may occur via the mechanism proposed for triglyceride synthesis in liver (J. Am. Chem. Soc., 78:3550, 1956). If this mechanism is correct, intestinal phosphatidic acids should become labeled when incubated in either C^{14}-palmitic acid or $NaH_2P^{32}O_4$. Everted intestinal segments from the hamster were thus incubated and the various phospholipids were chromatographically separated. The predominant activity was present in a fast moving spot (Rf .92) which corresponded to known phosphatidic acids. This spot was not detectable with rhodamine 6G staining which readily visulized the remaining phospholipids. The P^{32}-labeled phosphatidic acids were eluted and demonstrated to yield α-glycerophosphate. The incorporation of P^{32} into phosphatidic acids was increased 2 to 3 fold when incubated in the presence of fatty acids. This increase in phosphatidic acids was not paralleled by a significant increase in the remaining phospholipids. (Supported by grant No. A-3108, U. S. Public Health Service.)

12 *Nutrition*

TURNOVER RATES OF PLASMA NONESTERIFIED FATTY ACIDS IN THE POST ABSORPTIVE DOG. Margaret W. Bates * and Robert E. Olson, Dept. OF Biochem. and Nutr., Univ. of Pittsburgh, Pittsburgh, Pa.

The turnover rate of plasma nonesterified fatty acids (NEFA) has been found to be very rapid ($t_{\frac{1}{2}}$=2-3 minutes) in a variety of common experimental animals with albumin-palmitate-1-C^{14} (Fredrickson and Gordon, Physiol. Rev., 38, 585 (1958)). In the studies to be reported the turnover of NEFA was measured in the postabsorptive dog with two tracers. The first was made artificially in the usual way by labeling plasma in vitro with palmitate-1-C^{14}. The second was made physiologically by feeding a dog palmitate-1-C^{14} and obtaining samples of plasma 24 hours later. The tracers were introduced into recipient dogs by cross circulation or by intravenous injection and the plasma NEFA fraction isolated by silicic acid chromatography and counted. When labeled physiological NEFA were used, the total rates of appearance (R_a) and total rates of disappearance (R_d) were 0-4.5 mg/kg/hr. When labeled synthetic NEFA were used as the tracer, R_a and R_d of 63-296 mg/kg/hr were initially found. After 60 minutes R_a and R_d were 2.9 and 2.1 mg/kg/hr, similar to the rates obtained with labeled physiological NEFA. The large initial turnover rates obtained with the synthetic NEFA may reflect selective removal of this tracer by animal tissues. Recycling does not appear to explain the low turnover rate of physiological NEFA.

149. LIPIDS–Lipoproteins

1 *Physiology*

RESTITUTION OF α-LIPOPROTEINS IN DOG PLASMA FOLLOWING INJECTION OF THE LIPID-FREE PROTEIN MOIETY. A. Scanu and W. L. Hughes (intr. by L.A. Lewis.) Research Div., Cleveland Clinic, Cleve., O. and Med. Dept., B.N.L., Upton, L.I., N.Y.

Lipoproteins of d 1.063-1.21 (αLP) from normal dog sera were freed of lipids as previously described (Arch. Biochem. Biophys. 74:390, 1958). The protein thus obtained (αP) sedimented at d 1.21 and had properties of a native protein. Both αLP and αP were labeled with I^{131}. Male dogs, while on a constant diet with 10 drops of Lugol's solution/day, were injected IV with tracer amounts of αLP-I^{131}. This protein disappeared from plasma at a constant rate (half-time: 3.5 days). About 2 months later the same dogs were given, IV, αP-I^{131}. Rate of disappearance and half-time were the same as with αLP-I^{131}. In both experiments the radioactivity was contained in the d 1.063-1.21 lipoproteins; no counts were detected in the low-density class and chylomicrons. Data indicate that αP recombines in vivo with serum lipids to restore a native αLP. These results are consistent with our in vitro findings which showed formation of αLP-I^{131} by equilibrating αP-I^{131} with serum of αLP (Symp. Prot. Fd., Boston, 1959). The lack of exchange of αLP with low-density lipoproteins and chylomicrons is in agreement with the data of Gitlin et al. (J. Clin. Invest. 37:172, 1958). (Supported by grants of U.S. Atom. Energy Comm., U.S. Public Health and Cleveland Area Heart Society.)

2 *Physiology*

SIMILARITIES IN STRUCTURES OF HUMAN SERUM LIPOPROTEINS. B. Shore and V. Shore (intr. by G.M. Schoepfle), Physiol. Dept., Washington U. Med. School, St. Louis, Mo.

The distribution of N-terminal aspartic and glutamic acids, serine, and threonine, and electrophoretic mobilities (u) of -3.9-4.0 Tiselius units in pH 8.6, 0.1μ veronal suggest a possibly similar structure in D<1.007 (S_f20+), D 1.007-1.019 (S_f12-20) and D 1.045-1.065 (S_f1.5-2.5) lipoproteins (lps). The u of S_f 20-200 lps after addition to serum (for density stability) is -3.6 in the veronal, and -4.0 after suitable viscosity correction. The u in 8.6 veronal of -6.25 for the D 1.090-1.105 ($S_{20,w}$ (0.3%) = 4.94) lps and u of -6.4 for the D 1.140-1.153 ($S_{20,w}$ (0.3%) = 4.78) lps, their N-aspartic acid-C-threonine chain per 40,000 gms protein, and their apparent lack of -SH groups, suggest a possibly similar structure in these lps. The C16-18 NEFA content of the high density lps is probably reflected in their u's. In the D 1.028-1.033 (S_f6-8) lps, the u is -3.2 in the 8.6 veronal, while glutamic acid is the principal, serine and threonine minor, and aspartic acid a lesser N-terminal amino acid. (Supported by U.S.P.H.S. grant H-3629.)

3 *Physiology*

In Vitro Alterations of Molecular Composition of Serum Lipoproteins by Non-ionic Detergents. T.T.Tsaltas and L. M. Tocantins, Cardeza Foundation, Jefferson Medical College, Philadelphia, Pa.

Non-ionic detergents Tween 40,80 produce alterations in electrophoretic mobility of lipoproteins attributed to the attachment of Tween to lipoprotein molecules as demonstrated by radioisotope studies (T.Tsaltas, Fed.Proc.17:325,1958). Ultracentrifugal and electrophoretic changes can be invariably produced by adding 0.7% Tween to the serum and incubating at 37°C for 2-3 hours. Incubation is essential. At room temperature, 36 hours are required to produce similar changes while storage of the Tween-serum mixture at 0-4°C prevents their appearance. Prolonged dialysis of the incubated mixture fails to reverse the changes. A marked increase of the lipoproteins floating at D≥1.063 along with 5-10 fold increase of the cholesterol and phospholipid content of the top fractions was observed after preparative ultracentrifugation of incubated Tween-serum mixtures, followed by chemical analysis. A 3-4 fold increase of the non-protein moiety of these lipoproteins was also found. Analytical ultracentrifuge patterns showed a shift to Sf values lower than the controls as well as the appearance of new peaks with very low Sf values. The manner of production of these changes and the results obtained suggest participation of an enzymatic system, possibly a lipoprotein lipase

4 *Biochemistry*

INCREASED NET SYNTHESIS OF PLASMA LOW DENSITY LIPOPROTEINS BY THE PERFUSED LIVER OF NEPHROTIC RATS. Julian B. Marsh. Graduate School of Medicine and School of Medicine, University of Pennsylvania, Phila., Pa.

In previous studies (Marsh, J.B. and Drabkin, D. L., J. Biol. Chem. 230, 1083, 1958) an increased incorporation of acetate-1-C^{14} into liver and plasma cholesterol and fatty acids was shown to accompany the early stages of experimental nephrosis inthe rat. These findings supported the conclusion that increased hepatic lipogenesis is responsible for the development of nephrotic hyperlipemia. By use of the immunochemical method, net synthesis of plasma low density lipoproteins has been demonstrated previously in the isolated perfused rat liver. In 5 experiments with edematous nephrotic rats on the third day of the disease, an average increase of 250% in the rate of synthesis of the cholesterol of plasma lipoprotein in the perfusate was found in comparison with 7 control rats. Albumin synthesis was also increased by 55%. These experiments provide direct evidence for an increased rate of synthesis of plasma lipoprotein by nephrotic rats as the underlying cause of the hyperlipemia.

5 *Biochemistry*

THE FADING OF SUDAN BLACK B BOUND TO LIPOPROTEINS. Hugh J. McDonald and Leonard J. Banaszak*. Dept. of Biochem., Stritch Schl. of Med. and Grad. Schl., Loyola University, Chicago

The rate of fading of Sudan Black B (SBB) bound to high density (HD) and low density (LD) human serum lipoproteins was observed to be different on paper chromatograms and in aqueous solutions. Hydrogen peroxide (1% aqueous buffer) accelerates the fading. The changes produced in SBB and in the lipoprotein fractions following the peroxide treatment were reflected in the absorption spectra. Stained LD lipoproteins faded at a faster rate than HD lipoproteins; the color of the SBB bound to LD lipoproteins fades faster than does the dye itself when present in water containing 5% ethanol. SBB bound to lipoproteins in untreated serum does not fade under conditions similar to those which cause disappearance of color when the dye is bound to LD lipoproteins prepared by ultracentrifugation. Addition of small quantities of a serum protein preparation inhibits fading of SBB bound to LD lipoproteins prepared by ultracentrifugation. (Supported in part by a grant from the Chicago Heart Association).

6 *Biochemistry*

HYPERCHOLESTEREMIA AND ABSENCE OF ATHEROMA IN THE GERBIL. Samuel Gordon and Walter P. Cekleniak (intr. by P. H. Bell). Biochemistry Research Dept., Biochemical Research Section, Lederle Labs., American Cyanamid Co., Pearl River, N. Y.

Clarkson et. al. (Proc. Animal Care Panel 7:220, 1957) reported the absence of atheroma in hypercholesteremic gerbils. We have confirmed this and have determined the ultracentrifuge serum lipoprotein patterns of the gerbil during the development of hypercholesteremia. These were compared with those of the chicken, rabbit and man. It was found, that gerbils on a 1% cholesterol diet, develop a peak hypercholesteremia (1200 mg.%) within a week, from a pretreatment level of approximately 100 mg.%. This subsequently decreases after 30-60 days to an elevated plateau of 600 mg.%. Gross examination of the intimal surface of the aortas of these animals, with the aid of a low power microscope, showed no evidence of plaque formation. Maximum concentration of serum lipoproteins were found in the S_f 35-100 and S_f 100-400 fractions, with relatively low levels in the S_f 0-10, 10-20 and 20-35 fractions. In the hypercholesteremic chicken and rabbit and in the normal man the reverse was noted. These studies indicate a unique serum lipoprotein pattern in the hypercholesteremic gerbil in which there was an inverse relationship between concentration and density.

7 *Biochemistry*

ACTIVATION OF LIPOPROTEIN LIPASE BY POLYANIONS OTHER THAN HEPARIN. P. Bernfeld and T. F. Kelley*. Bio-Research Institute, Cambridge 41, Mass.

The in-vitro activation of lipoprotein lipase has been observed only with a few macromolecular polyanions, particularly heparin. In order to study the structural requirements for the activator, 32 different sulfated polysaccharides were tested for their potency to activate mouse heart lipoprotein lipase. Enzyme activity was measured by determining glycerol liberation. The presence of N-sulfate groups in the polysaccharide was determined by measuring the Van Slyke free amino nitrogen before and after mild hydrolysis. It was found that all polysaccharides with one or more sulfate groups per repeating unit, not containing hexosamine, were more or less potent inhibitors, independently of their molecular weight. Sulfated, N-acetylhexosamine containing polysaccharides were observed to exert little or no effect. The only significant activators of lipoprotein lipase appeared to be N-sulfate containing polysaccharides, e.g. heparin as well as deacetylated and subsequently sulfated chitin. These results suggest that the sulfamic acid groups contribute significantly to the ability of polyanions to activate lipoprotein lipase.

8 *Biochemistry*

DISTRIBUTION OF PROTEOLIPIDS IN THE HUMAN NERVOUS SYSTEM. Luigi Amaducci (Intr. by J.Folch). McLean Hospital, Waverley, and Harvard Medical School, Boston, Mass.

Since proteolipids are found in mammalian brain at 30-fold their concentration in peripheral nerve (J. Neurochem. 1:256, 1957; Exp. Cell Res. Suppl. 5:58, 1958), it seemed worthwhile to establish their distribution in various parts of the human nervous system. Tissue samples were obtained at autopsy (12-24 hrs. after death) from 30 anatomical areas of the nervous system of cases without manifest neurological disease. Washed total lipid extracts (J. Biol. Chem. 226:497, 1957) were analyzed for α-amino acid N before and after acid hydrolysis and the proteolipid protein content computed by multiplying the difference by the empirical factor 9. The results obtained show that although proteolipids are more abundant in central white matter (and optic nerves) than in gray matter, nevertheless, the concentration in each type of tissue varies markedly from one anatomical area to another. Thus, it is 2.7-3.0 mg/per gram fresh tissue in frontal cortex, and 5.6 mg. in occipital; in white matter, it is more abundant in cerebrum than in spinal cord and in the latter, the distribution is not uniform. The concentration in spinal roots (anterior, 4.4 mg; posterior, 4.7 mg) is less than in spinal cord but more than in sciatic nerve (1.2 mg). The proteolipid concentration does not consistently follow that of the total lipids. (Supported by Grant B-130, Natl. Insts. of Health).

9 *Biochemistry*

FLUORESCENT LIPIDS FROM HEART AGE PIGMENT. A. S. Mildvan* and B. L. Strehler. Gerontology Branch, National Heart Institute, and Baltimore City Hospitals, Baltimore 24, Maryland.

Previous work from this laboratory (J. Geront. 14, 430) has demonstrated a linear accumulation with age of a yellow-orange fluorescent particulate component in the human myocardium approximating 6-10% of the cell volume at advanced age. These particles were isolated by differential centrifugation (4600 x g) of sucrose homogenates of ventricular muscle of aged humans. Further purification was achieved by washing with sucrose and KCl solutions and/or by sedimentation through density gradients (particle density = 1.18 ± 0.03). Such preparations showed occasional blue-fluorescing impurities. A lipid extraction of lyophilized particles removed 29% of the weight and yielded a highly fluorescent solution. Silicic acid chromatography of this mixed lipid revealed a pale blue fluorescent band in the cholesterol ester fraction and a single bright yellow-orange fluorescent band moving with the cephalin fraction. We have observed that authentic solid cephalins and lecithin acquire an apparent yellow-orange fluorescence when undergoing autooxidative rancidity. We have been unable to distinguish differences between the infra red spectra of such a cephalin and that of our fluorescent lipid. The residue (71%) is brown, 16% nitrogen (80% of which is ninhydrin positive after hydrolysis) and possesses infra red peaks characteristic of protein.

10 *Nutrition*

LIPIDE DISTRIBUTION IN EGG YOLK LIPOPROTEIN COMPLEXES. Robert John Evans and Selma L. Bandemer*, Department of Agricultural Chemistry, Michigan State University, East Lansing, Michigan.

Egg yolk contains two lipoprotein complexes, lipovitellin and lipovitellenin. Lipovitellin and lipovitellenin were isolated and freeze dried. The lightly-bound lipides were extracted with petroleum ether and the tightly-bound ones with ethanol. Lipides were separated into neutral fats, phosphatidylcholine and phosphatidylethanolamine by chromatography on silicic acid. The fatty acids of each lipide were determined by gas-liquid chromatography. Lipovitellin contained 47.5% of the egg yolk protein and 6.8% of the lipide. Lipovitellenin contained 38.6% of the protein and 93.2% of the lipide. Lipovitellin contained 21.7% lipides, mostly tightly-bound. Lipovitellenin contained 82% of lipides, mostly lightly-bound. The lightly-bound lipide fraction was nearly all neutral fats. The tightly-bound lipide of lipovitellin was principally phosphatidylcholine and of lipovitellenin phosphatidylethanolamine. The neutral fats contained mainly palmitic and oleic acids; phosphatidylcholine mostly palmitic with smaller amounts of stearic and oleic; and phosphatidylethanolamine, palmitic, stearic and oleic acids.

XUM

150. LIPIDS–Phospholipids

1 *Physiology*

PHOSPHOLIPIDS OF THE AMERICAN COCKROACH. A. N. Siakotos and H. S. Zoller (intr. by C. C. Hassett) U. S. Army Chemical Warfare Laboratories, Army Chemical Center, Md.

Phospholipids from the American cockroach were fractionated by silicic acid column chromatography. These fractions were rechromatographed on silicic acid treated paper (Marinetti, 1958) and the number of phospholipids estimated in each column fraction. The fractions were analyzed for phosphorus, total nitrogen, amino nitrogen, carbohydrate, aldehyde, and ester groups. Confirmation of the identity of the individual components was carried out as follows: 1) the fractions were rerun on silicic acid treated paper and various qualitative tests applied; 2) these findings were checked with phospholipids isolated from other animal sources. The phospholipids isolated and identified by these procedures were phosphatidic acids, phosphatidyl serine, phosphatidyl ethanol amine, phosphatidyl choline, phosphoinositide, cerebrosides, sphingomyelin, and plasmalogen. The tissue distribution of these lipids and their alterations in composition during the process of molting were investigated. At the same time their relative stability was followed by measuring the rate of P^{32} incorporation and turnover. The metabolic significance of these findings will be discussed.

2 *Physiology*

PHOSPHOLIPID COMPOSITION OF HUMAN BRAIN TUMORS. Som N. Nayyar Richard E. McCaman and Robert F. Heimburger (intr. by Leslie W. Freeman). Section of Neurosurgery and Inst. of Psychiatric Research, Indiana Univ. Med. Center, Indianapolis, Indiana.

Surgical specimens of primary intracranial neoplasms were analyzed for cephalin, lecithin and sphingomyelin by the method of Robins et. al. (J. Biol. Chem. 220: 66, 1956) and for DNA by the method of Kissane and Robins (Ibid 233: 184, 1958). The number of cells in the sample analyzed was calculated from the DNA content by using the values reported by Heller and Elliot (Canad. J. Biochem. Physiol. 32:584, 1954). Surgical and autopsy specimens of grossly normal cortex and white matter were used for comparison. Total lipid-P content of various tumors, on the basis of dry weight, was found to vary from approx. 25 to 80% of that of normal cortex and about 15 to 50% of that of white matter. Cephalin content of normal tissues was about 2 to 4 times as great as that of lecithin, whereas, in most of the tumors, the concentration of these two lipids was practically equal and in a few tumors lecithin predominated. The amount of various lipid fractions per tumor cell was found to be 4 to 10% of the corresponding values for normal cortex.

(Supported by USPHS grant C4650 and Institutional Cancer grant).

3 *Biochemistry*

LOMBRICINE AND SERINE ETHANOLAMINE O-PHOSPHODIESTER IN THE EARTHWORM. R.J. Rossiter, T.J. Gaffney*, H. Rosenberg* and A.H. Ennor*. Dept. Biochem., Australian Natl. Univ., Canberra.

Previously it was suggested that serine ethanolamine O-phosphodiester (SEP) was the biological precursor of lombricine (O-phosphodiester of serine and guanidinoethanol), the N-phosphoryl derivative of which is the phosphagen of the earthworm. Further support for this conclusion was the subsequent isolation of SEP from the earthworm and the demonstration that the constituent serine, like that of lombricine (Nature, 183, 591, 1959), had the unusual D-configuration. This is in contrast to the demonstration of L-serine in SEP isolated from turtles and other species (Roberts and Lowe, J.B.C., 211, 1, 1954). After the oral administration of inorganic P^{32}, ethanolamine-1,2-C^{14}, or D,L-serine-3-C^{14} to earthworms, both SEP and lombricine were found to contain radioactivity. In muscle and gut the specific radioactivity of SEP was much greater than that of lombricine. On the other hand, after the administration of amidine labelled arginine-C^{14} there was negligible radioactivity in SEP and considerable activity in lombricine, almost all of which was in the guanidinoethanol portion of the molecule. These findings strongly suggest that in the earthworm SEP is the precursor of lombricine and that the amidino group of lombricine is derived from arginine by a transamidinase-catalysed reaction.

4 *Biochemistry*

PLASMALOGENS OF MARINE INVERTEBRATES. Maurice M. Rapport and Nicholas F. Alonzo*. Albert Einstein College of Medicine, New York, and Marine Biological Laboratory, Woods Hole.

Recent reports indicated that plasmalogens of marine animals (sea anemone, crab) differ in two important respects from those in most mammalian tissues; namely, that they have an acetal rather than an α,β-unsaturated ether structure, and that they have choline as the predominant base rather than ethanolamine. We have therefore studied the lipids of Arbacia, Asterias, Busycon, Limulus, Loligo, Metridium, Mya, Mytilus, Pecten, Thyone, and Venus. Total lipid extracts were analyzed by specific iodination and p-nitrophenylhydrazone formation (Rapport and Lerner, Biochim. Biophys. Acta 1959:33, 319). Fractionation on silicic acid at 5°C was then used to separate ninhydrin-positive phosphatides from those containing choline. The lipids of these species, excepting Loligo, contain large quantities of plasmalogens which have the α,β-unsaturated ether linkage in their aldehydogenic chains. The plasmalogens are found principally in the "cephalin" fraction. The structure and distribution of this type of phosphatide are therefore similar in both mammalian and marine invertebrate tissues.

5 *Biochemistry*

MECHANISM OF CHOLINE BIOSYNTHESIS. Jon Bremer* and David M. Greenberg. Univ. of California School of Med., San Francisco.

We have recently shown that choline-C^{14} can be isolated from the phospholipids of rat liver microsomes after incubation with S-adenosylmethionine-$C^{14}H_3$. Mono- and dimethylaminoethanol were also isolated (Bremer and Greenberg, Biochim. et Biophys. Acta, in press). The radioactive choline-containing phospholipid has now been identified as lecithin. When chromatographed on a silicic acid column the choline radioactivity coincided exactly with the lecithin phosphorus peak. When the isolated phospholipids were treated with Clostridium welchii toxin according to Hanahan and Vercamer (J. Am. Chem. Soc., 76, 1804 (1954)), radioactive phosphocholine was isolated. The pH optimum of the reaction is at 9 or possibly higher. Choline synthesis is stimulated by BAL, but glutathione, cysteine, ascorbic acid or versene are without effect. Mercaptoethanol is strongly inhibitory. The microsomes may be lyophilized and treated with n-butanol and acetone without appreciable loss of activity. So far, no increase in choline formation has been obtained by the addition of synthetic or natural cephalin, presumably due to the high phospholipid content of the microsomes.

6 *Biochemistry*

PHOSPHOLIPIDS IN SPINAL FLUID AND BRAIN. N. M. Papadopoulos* W. Cevallos* and W. C. Hess. Georgetown Univ. School of Medicine, Wash. D. C.

Pooled human spinal fluid was extracted with chloroform-methanol. From the dried lipid extract the phospholipids were separated and determined by the method of Philipps (Biochim. and Biophys. Acta, 29, 594, 1958), used in blood. The phospholipid fractions from the column were chromatographed on silicic acid impregnated paper. Cephalin, lecithin, sphingomyelin and lysolecithin were identified. While these phospholipids have also been found in blood the distribution pattern differs from that in spinal fluid. These results confirm our previous findings of the presence of cephalin, lecithin, and sphingomyelin in spinal fluid, and establish for the first time the presence of lysolecithin. The average amount of total phospholipid found was 0.481 mg/100 ml. and the values for cephalin, lecithin, sphingomyelin and lysolecithin were 0.082, 0.290, 0.077, and .032 respectively. Lipids from gray and white matter of human brain were extracted by the procedure of Folch et al (J. Biol. Chem. 226, 497, 1957), and chromatographed on a silicic acid column. The results revealed the presence of all four phospholipid components found in blood and spinal fluid but a different distribution in gray and white matter. The relation of these phospholipids to those in blood and spinal fluid will be discussed.

7 *Biochemistry*

AN ENZYMATIC REACTION OF CDP-DIGLYCERIDE WITH L-ALPHA-GLYCEROPHOSPHATE. John Kiyasu,* Henry Paulus* and Eugene P. Kennedy. Dept. of Biochemistry, University of Chicago.

Enzyme preparations from liver have been found to catalyze a reaction between CDP-dipalmitin and L-alpha-glycerophosphate, during the course of which CMP is released and C-14-labeled glycerophosphate is converted to a lipid. Free glycerol does not react with CDP-dipalmitin in this system, suggesting that the primary products of the reaction are phosphatidyl-glycerophosphate and CMP. Experiments with P-32-labeled L-alpha-glycerophosphate suggest that the phosphatidyl-glycerophosphate may be subsequently dephosphorylated to yield phosphatidyl-glycerol.

8 *Biochemistry*

FURTHER STUDIES ON THE FORMATION OF PHOSPHATIDYL DIMETHYLETHANOLAMINE IN LIVER PREPARATIONS. Camillo Artom. Bowman Gray School of Medicine, Winston-Salem, N.C.

Previous findings on the incorporation of C^{14}-labeled dimethylethanolamine(DME) into the phospholipids of rat liver slices and homogenates(Crowder and Artom, Fed.Proc.11:199, 1952) were confirmed and extended. Both mitochondria and supernate are still active. The activity of all preparations is increased by addition of cytidine derivatives(CMP, CDP, CTP), and is strongly inhibited by non-isotopic choline. Phosphatidyl DME was characterized by chromatography of the intact phospholipid on silicic acid, and by steam distillation (Artom and Crowder, Fed.Proc. 8:180, 1949) and paper chromatography of the products of total, or partial, hydrolysis. In the incubation with liver slices, phosphatidyl choline also becomes extensively labeled. Experiments in which, after an initial period of incubation, further incorporation of the isotope was inhibited by addition of non-labeled choline, or DME, suggest that, especially in the presence of methionine, at least some phosphatidyl choline was formed by methylation of the DME moiety of phosphatidyl DME. This concept would be in line with the findings of Bremer and Greenberg(Biochem.Biophys.Acta, 35:287, 1959) on intact rats receiving labeled methionine. (Aided by AEC Contract AT-40-1-(1638) and U.S.P.H.grant A-754)

9 *Biochemistry*

2-AMINO-2-METHYLPROPANOL, A COMPETITIVE INHIBITOR OF CHOLINE SYNTHESIS. Ibert C. Wells and Charles N. Remy. Biochem. Dept. State Univ. of N. Y. Med. Coll. and V. A. Hosp., Syracuse.

Previous studies in this laboratory demonstrated an overall inhibition of choline synthesis from methionine-CH_3-C^{14} in rats treated with 2-amino-2-methylpropanol (MAP). In these experiments the C^{14} was found only in the methyl groups of choline. Present experiments indicate the site of inhibition as a competition between MAP and dimethylaminoethanol (DMAE). Weanling rats, treated as previously described (Fed. Proc. 18, 1836 (1959)), were injected I.P. with L-methionine-CH_3-C^{14} plus DMAE or with DMAE-1,2-C^{14} plus L-methionine and the specific activity of liver and kidney lipide-bound choline determined after 2 hrs. Both types of experiment revealed that the inhibition of choline synthesis by MAP could be progressively reversed by increasing concentrations of DMAE. Lineweaver-Burk plots, typical of competitive inhibition, were obtained from experiments in which liver slices from MAP treated animals were incubated with MAP and DMAE-1,2-C^{14} in Krebs phosphate. These results indicate that the locus of MAP inhibition of choline synthesis from methionine is at the DMAE stage or at a stage metabolically closer to choline rather than at the ethanolamine stage. (Supported by grant A-3192 MET, U.S.P.H.)

10 *Biochemistry*

SILICIC ACID CHROMATOGRAPHY OF HEART PHOSPHATIDES. Hermann Bader and H. E. Morgan. (intro. by C. R. Park) Physiol., Vanderbilt, Nashville.

Previous methods of silicic acid chromatography have been modified. Lipids of rat heart muscle were extracted at 0° from frozen powders using chloroform-methanol (2:1) with $MgCl_2$(2mM). The lipids were adsorbed on freshly prepared silicic acid columns and eluted with 500 ml chloroform mixed with 175 ml methanol so that a continuous concave-upward gradient was achieved. The separation was highly reproduceable with respect to quantity and distribution of lipid P and was free of artifactual peaks seen with earlier stepwise elution procedures. The 100 uMoles of lipid P applied to the column separated into 5 peaks characterized as follows. 16uM of non-nitrogen containing phosphatide appeared in the first peak, 2uM of amino nitrogen containing phosphatide in the second, 40uM of phosphatide with an amino nitrogen:P ratio of 1:1 in the third, 2uM of phosphatide in the fourth, and 40uM of phosphatide with a choline:P ratio of 1:1 in the fifth peak. Recovery was 100%. The pattern was considerably modified by omission of $MgCl_2$.

11 *Biochemistry*

SEPARATION OF PHOSPHATIDYLETHANOLAMINE (PE) AND PHOSPHATIDYLSERINE (PS). **George Rouser, A.J.Bauman*, John O'Brien*, and Dorothy Heller*. Dept. Biochem., City of Hope Med. Center, Duarte, Calif.**

PE + PS recovered from a conventional silicic acid (SA) column can be separated on a silicic acid-silicate (SAS) column prepared by passing $CHCl_3/CH_3OH$/aqueous NH_3(C/M/NH_3) through a SA column. PE is eluted with C/M 4/1 and PS with M. PE and PS are separable using the acetate form of DEAE cellulose. PE has been eluted with C/M mixtures or M. PS was not elutable. Phosphocellulose (PC) containing water can be used for the separation and recovery of PE and PS. C/M mixtures are used for elution. As the water content of PC increases the proportion of methanol must be increased. The separation on DEAE appears to depend in part upon ion exchange, while the separations on SAS and PC columns appears to depend upon hydrogen bonding of the anionic column component through water to the carboxylate ion (PS) and to a lesser extent to phosphate and amino groups (PE). Separations and sample storage must be carried out in the absence of oxygen as PE and PS are readily oxidisable. Oxidative change in samples prior to chromatography must be avoided as some early oxidation products may emerge with, or near to, the parent compounds on SA columns, although these altered compounds appear to be retained by DEAE.

12 *Biochemistry*

CONVERSION OF 2-DIMETHYLAMINOETHANOL TO DIMETHYLGLYCINE IN LIVER. Gary Small* and Warwick Sakami, Dept. of Biochem., Western Reserve U., Cleve., O.

Conversion of 2-dimethylaminoethanol-1,2-C^{14} to dimethylglycine-C^{14} has been demonstrated to occur in a rat liver extract prepared by centrifugation of a pH 7.8 phosphate buffer homogenate at 80,000 X g for 1 hr. Dimethylglycine-C^{14} was isolated from incubation mixtures by chromatography on Dowex 50 eluted with 1 N HCl: the identity of the product was established by paper chromatography with 80% pyridine. Methylene blue stimulates the conversion and phenazine methosulfate inhibits. Treatment of the enzyme preparation with Dowex 1 (Cl^-) and dialysis results in decreased activity which is increased above the original level by DPN. Maximum conversion is 1 µmole per hr per ml of extract (from 0.22 g liver). Addition of choline produces a small decrease in dimethylglycine-C^{14} activity. Choline-C^{14} is not converted to dimethylglycine-C^{14}. Dimethylaminoethanol-C^{14} + choline-C^{14} produce the same amount of dimethylglycine-C^{14} as dimethylaminoethanol-C^{14} + choline-C^{12}. Sarcosine-C^{14} is not formed in this system.

XUM

13 *Biochemistry*

LIPID ETHANOLAMINE AND SERINE IN ANIMAL TISSUES.
J. M. McKibbin. State Univ. of New York, Syracuse, N.Y.
Lipid extracts of animal tissues were prepared and purified to minimize the content of peptides. The purified lipid equivalent to 3 mM N was saponified at 21-23 deg.C. with 1 N NaOH for 3 days, acidified and extracted with chloroform. The aqueous phase contained 60-80 % of the total lipid N as free base. The base mixture was divided into 5 fractions on Dowex 50 Na. Ethanolamine, choline, and monoamino acids were separated exclusively in three of these and minor components were present in the other two. All fractions were analyzed for total N, P, choline, amino N, periodate active N and NH_3, and were further examined by paper chromatography. The alkali-stable lipids in the chloroform phase were analyzed similarly after more drastic hydrolysis procedures. A fairly complete identification of the lipid N was possible by summing the individual bases in the six fractions. Extracts of dog heart, lung, liver, kidney, pancreas and intestine contained amino acids other than serine and in total amount comparable to the serine present. Ethanolamine N comprised 15.3-24.6 % of lipid N and serine N 2.5-8.7 % in these tissues. The latter values are much lower than those reported previously.
(Supported by The Life Insurance Medical Research Fund).

14 *Biochemistry*

SOME PRECURSORS OF BRAIN INOSITOL PHOSPHATIDE.
W. Thompson*, D. Subrahmanyam* and K. P. Strickland. Dept. of Biochemistry, University of Western Ontario, London, Canada.
Previous work with H^3-inositol indicated that CTP and phosphatidic acid stimulated labelling of phospholipids by water homogenates of rat brain (Thompson, Strickland and Rossiter, Fed. Proc. 18, 338, 1959). P^{32}- or C^{14}-L-α-glycerophosphate (L-α-GP) was incorporated into the phospholipids of brain homogenates in the presence of ATP and CoA. Paper chromatographic separation of the water-soluble products obtained on mild alkaline hydrolysis or separation of the phospholipids on silicic acid impregnated paper revealed that the radioactivity was present mainly in inositol phosphatide and phosphatidic acid. The incorporation of L-α-GP into inositol phosphatide was slightly stimulated by CTP and greatly stimulated by CDP-choline while the labelling of phosphatidic acid was depressed. The P^{32}- or H^3-labelled brain inositide was chromatographically similar to liver phosphatidyl inositol and on hydrolysis in 6N HCl yielded a product which was electrophoretically identical to inositol monophosphate. The C^{14}-label from the glycerol portion of phosphatidic acid (prepared enzymatically from C^{14}-lecithin) was incorporated into brain inositol phosphatide in the presence of CTP. L-α-GP and phosphatidic acid are thus precursors of brain inositide but may be incorporated by alternative pathways requiring different co-factors.

15 *Biochemistry*

THE PLASMALOGENS OF INVERTEBRATES.
M. H. Hack and A. E. Gussin*. Departments of Medicine and Anatomy Tulane Univ. New Orleans, La.

Twenty species of invertebrates representing six phyla (Protozoa to Mollusca) were examined for their plasmalogen pattern by the paper chromatographic technique previously described (Hoppe-Seyler's Z. f. physiol. Chemie 315, 157 (1959). The total lipid was extracted from the entire animal by chloroform-methanol (20 ml per gram wet tissue), 25-100 µl of extract were suitable for chromatography. Ethanolamine plasmalogen was relatively most abundant in the protozoa and in the flat and round worms and more nearly equal to the choline plasmalogen in the remaining phyla. Only in the molluscs (3 species) was serine plasmalogen observed. It was readily isolated by Folch's procedure (J. Biol. Chem. 174, 439 (1948) and the serine identified by one and two dimentional paper chromatography. By the same procedure serine plasmalogen of vertebrate brain was readily demonstrated.

Supported by grants H 2564 and HTS 5133 USPHS

16 *Biochemistry*

FORMATION OF PALMITALDEHYDE IN RAT BRAIN. Keiichiro Hoshishima*, Pierre V. Vignais*, and Irving Zabin. Dept. of Physiological Chemistry, University of California, Los Angeles.
Although the existence in animal tissues of higher fatty aldehydes has been recognized for many years, little information is available concerning their metabolism. Ehrlich and Waelsh and Korey have stated that higher fatty acids are transformed into higher fatty aldehydes. The authors isolated the enzyme which converts palmityl-CoA (Palmityl-GSH) into palmitaldehyde from brains of young female rats.
Homogenized rat brains in sucrose-versene solution were centrifuged at 20,000 RFG and the supernatant containing the so-called small microsomes was mixed with equal volumes of 0.012M Na-desoxycholate and 1M glycine and left for 10 minutes at 2°C. To remove the cell debris, the mixture was centrifuged at 100,000 RFG for 60 min. The active enzyme was precipitated with 1 1/2 volumes of cold acetone at -20°C.
The activity of the purified enzyme was about 40 times that of the original homogenate based on protein content. The optimum pH for this enzyme was pH 6.5-7.25. The enzyme required DPNH; TPNH did not substitute. The enzymatic activity was inhibited by p-chloromercuribenzoate and monoiod-acetate. The inhibition was reversed with the addition of GSH. Palmitic and Stearic Co-A were the best substrates.
Supported by N.H.I. grant.

17 *Pharmacology*

EFFECT OF PARASYMPATHOMIMETIC STIMULATION ON THE METABOLISM OF PHOSPHATIDO-PEPTIDE. C.G.Huggins (intr. by R.M. Isenberger). Dept. of Pharmacology, Kansas Univ. Med. School, K.C., Kansas.
The question of whether the metabolism of phosphatido-peptide phosphorus is related to secretory activity in the pancreas has not been established. Accordingly, slices were prepared from chicken pancreas and incubated in Warburg vessels containing varying concentrations of acetylcholine (10^{-7} to 10^{-2}M), physostigmine (3×10^{-4}M) and 30 µc of $Na_2HP^{-32}O_4$. It was found that maximal stimulation of the turnover of phosphorus in the phosphatido-peptide fraction occurred in the presence of 10^{-4}M acetylcholine. Similar results were obtained with the phospholipid fraction. Under these conditions there was no significant effect on the metabolism of phosphorus in the organic acid-soluble phosphorus fraction. Maximal secretion of amylase by the slice occurred in the presence of 10^{-6} to 10^{-5}M acetylcholine. The stimulatory effect of acetylcholine (10^{-4}M) was inhibited approximately 75% by atropine (10^{-4}M). These phenomena were also investigated in the pancreas of the intact animal (white mice) using pilocarpine as the cholinergic agent. Essentially the same results were obtained with the phosphatido-peptide fraction and the other phosphorus-containing fractions as were found in the above experiments. It is concluded that secretory processes in pancreas are related to the metabolism of phosphorus in the phosphatido-peptide fraction.

18 *Pharmacology*

ORIGIN OF THE METHYL GROUPS OF CHOLINE. J.D. Wilson*, K.D. Gibson*, and S. Udenfriend. Natl. Heart Inst., Bethesda, Md.
While it has been established that the methyl group of methionine is transferred directly to choline (duVigneaud, V., et al., J.A.C.S. 78: 5131, 1956) other work suggests that only one methyl group arises in this way, the other two coming from the one-carbon pool (Stekol, J.A., Am. J. Clin. Nutr. 6: 200, 1958). We have studied factors influencing phospholipid choline synthesis in rat liver slices. Unlabeled ethanolamine and formate did not affect incorporation of methionine-CH_3-C^{14} and unlabeled methionine and formate did not affect incorporation of ethanolamine-1,2-C^{14}. However unlabeled methionine consistently depressed incorporation of formate-C^{14}; the extent of this depression increased from 75% to 97% as methionine was increased from 5×10^{-4}M to 5×10^{-3}M. Furthermore, the specific activity of reisolated methionine paralleled that of choline. Homocysteine had a similar effect at high concentrations; however ethanolamine did not affect incorporation of formate-C^{14}. In the intact rat liver methionine depressed conversion of formate-C^{14} to phospholipid dimethylethanolamine and choline to the same degree. These results suggest that methionine is the immediate source of all methyl groups of choline. Further support for this hypothesis comes from preliminary observations on a particulate enzyme system of rat liver which synthesizes choline using S-adenosyl-methionine as sole methyl donor.

19 *Nutrition*

THE DETERMINATION OF PHOSPHATIDYL SERINE (PS) AND PHOSPHATIDYL ETHANOLAMINE (PE) BY GLASS PAPER CHROMATOGRAPHY. James E. Muldrey*, O. Neal Miller, James G. Hamilton*, Tulane Univ. Sch. of Med., New Orleans, La.

The chromatographic technique of Muldrey, Miller and Hamilton (J. Lipid Res. 1, 48 (1959)) has been improved to permit the quantitative estimation of microgram quantities of **PS** and **PE** in the presence of choline-containing and neutral lipids. It has been applied to serum and whole blood. Glass fiber paper impregnated with 2.4% sodium silicate solution is spotted with samples, dried, and developed in benzene to sweep neutral lipids with the solvent front. A second sweep in benzene-pyridine-water (BPW), 100/125/13, (v/v/v) leaves the **PS** at the origin and moves lecithin, sphingomyelin and **PE** up the paper. A third solvent, BPW, 100/125/25, moves the **PS** as a separate spot. The second and third solvents may be applied with an intervening drying in vacuum or simply, by transferring the paper directly from the second to the third solvent after the second solvent, (100/125/13), has traveled half-way up the paper. The dried papers are sprayed with concentrated sulfuric acid and heated at 230°C for 3 minutes. Densitometry of the charred spots shows a linear relationship between 0.2 and about 1.3 micrograms of **PE** or **PS**.

2c *Immunology*

THE ROLE OF PHOSPHOLIPIDES IN THE METABOLISM OF AMINO ACIDS BY MAMMALIAN CELLS. W. L. Gaby, H. L. Wolin* and Ihor Zajac*. Hahnemann Med. Coll. & Hosp., Phila., Pa. and Seton Hall Coll. of Med. & Dent., Jersey City, N. J.

Previous experiments have indicated that the phospholipides of the mold P. chrysogenum appear to play a role in the metabolism of amino acid. The study has been extended in an effort to ascertain the general nature of the phenomena and has included observations on amino acid incorporation by the phospholipides of rabbit liver slices and Ehrlich ascites tumor cells. Rabbit liver slices were incubated at 37° in vitro with DL-leucine or DL-tyrosine in phosphate buffer. Incorporation of labeled amino acids into Ehrlich ascites cells was accomplished by the intra-peritoneal injection of DL-leucine-1-C^{14}, DL-glycine-1-C^{14} or DL-lysine-1-C^{14} into mice bearing 6 or 7 day old ascites tumors. The presence of the amino acids in the phospholipides extracted from these cells could be demonstrated by paper chromatography in the water soluble fraction of an acid hydrolysate of the lipide complex. The labeled amino acids could not be separated from the phospholipides by electrophoresis. The phospholipides did not incorporate the amino acids in the absence of actively metabolizing cells.

1 *Biochemistry*

151. LIPIDS–Sterols

RADIOCHEMICAL PURITY OF H^3-β-SITOSTEROLS TRITIATED BY THE WILZBACH PROCEDURE AND BY EXCHANGE OVER PLATINUM DIOXIDE. Harold Werbin, I. L. Chaikoff,* and Miles R. Imada,* Dept. of Physiology, Univ. of California, Berkeley, Calif.

The radiochemical homogeneity of β-sitosterols tritiated by the Wilzbach procedure (W) and by catalytic exchange over PtO_2 (C) was compared. The specific activity (SA) of the chloride and epoxides of W showed that over 50% of the H^3 was not in the Δ^5-sterol. The SA of the same derivatives of C indicated that this sterol did not have more than 5% contamination. The finding of H^3-dihydrositosterol during chromatographic purification of the epoxides indicated that this saturated sterol was the principal contaminant. The dihydrositosterol contents of the labeled β-sitosterols and the unlabeled sterols from which they were prepared were determined. Tritiation by the Wilzbach procedure resulted in 9% reduction of the β-sitosterol and the saturated sterol contained 61% of the H^3. Tritiation by exchange over PtO_2 resulted in negligible reduction 2%, and the dihydrositosterol present had 8% of the H^3 of the sample. α-sitosterol epoxide acetate, m.p. 115.5-116.5°, β-sitosterol epoxide acetate, m.p. 148-149.5°, and a 1:1 complex of both, m.p. 139-142°, were separated by chromatography on Al_2O_3 and characterized.

2 *Pathology*

EFFECTS OF TOXIC FATS IN CHICKENS. James R. Allen (intr. by J. J. Lalich). Univ. of Wisconsin Med. School, Madison, Wis.

The unsaponifiable residue following distillation of certain fats caused the death of several million chickens in the U.S.A. during 1957. Toxic fat is a sterol; however, its chemical formula is not known. This fat will produce hydropericardium, ascites and death. The studies included determinations of protein loss in transudates and in addition the histology of the tissues. 100 birds were divided into 10 lots and fed a diet containing 0.5%, 1.0%, 3.0% and 5.0% toxic fat. Concentrations of 3 and 5% toxic fat produced death in less than 3 weeks. Birds survived when fed 0.5 or 1.0% toxic fat for 5 weeks. As the concentration of toxic fat in the diet was increased, growth rate and food consumption decreased. After the third week, test birds fed 1, 3 and 5% toxic fat developed hydropericardium and ascites. Hydropericardial fluid aspirated at autopsy varied from 0.5 to 5.0 ml. Ascitic volumes were not ascertained due to coagulation of the transudate. Hydropericardial fluid had an average level of 1.7± 0.7 gm % protein. Serum protein was usually reduced in birds with massive transudates. Histopathologic examination revealed focal hepatic necrosis and hemorrhage. (Supported by a grant from U.S.P.H., HF-8985.)

152. LIPIDS–Sterols–Blood Levels

1 *Physiology*

SOLUBILITY OF CHOLESTEROL IN HUMAN SERUM. Dale A. Clark and J. J. Franks (intr. by Richard W. Bancroft.) Pharmacology-Biochemistry Branch, School of Aviation Medicine, Brooks AFB, Texas.

If cholesterol is deposited in the arterial wall because of overloading of the transport capacity of the serum, it is important to measure the extent to which this capacity is normally utilized. Crystalline cholesterol was added to human serum and incubated for 4 hours at 37° C. with occasional shaking. The mixture was then filtered to remove undissolved cholesterol, and the concentration of cholesterol in the filtrate was determined by the method of Robinson and Pugh (U. S. Armed Forces Med. J. 9:501, 1958). Control samples to which no cholesterol was added were similarly incubated, filtered, and analyzed. When sufficient cholesterol was added to saturate the serum, the average increase in cholesterol concentration in the serum of a group of healthy normals was 0.26 mg./ml. To reach this saturation level, however, it was necessary to incubate each cc. of serum with about 40 mg. of crystalline cholesterol. Lipoprotein determination by the Gofman technique showed only minor variation in serum lipoproteins after saturation with cholesterol.

1 *Physiology*

CHEMICAL ESTIMATION OF PLASMA ESTROGENS. Harold J. Nicholas * and Rosemary Schrepfer * (intr. by N. J. Wade.) Dept. of Obst., Univ. of Kansas Med. Cntr., K. C., Kansas.

Efforts were directed toward estimating estrone, estradiol-17β and estriol in 10-15 ml. plasma samples without mineral acid hydrolysis as a preliminary step. An extraction and fractionation procedure was devised from standardized methods. This was coupled with quantitative determination of the steroid methyl ethers by fluorimetry after chromatography according to Brown (Biochem. J., 60, 185 (1955) (modified). For preliminary studies, late human pregnancy plasma was used. Some "free" estrogen was always extracted from fresh pregnancy plasma with ether and toluene. The remainder was "liberated" by allowing the plasma to incubate overnight at 37° under toluene. Occasionally glucuronidase and Mylase P gave higher values than obtained by incubating plasma alone, but this did not occur with all pregnancy plasma. Estriol was not always quantitatively most significant.

XUM

3 *Biochemistry*

CHOLESTEROL-LOWERING ACTION OF THE COMBINATION OF MK-135 AND BENZMALECENE IN COCKERELS AND DOGS. David M. Tennent, Gunther W. Kuron*, Mary E. Zanetti*, and Walther H. Ott.* The Merck Institute for Therapeutic Research, Rahway, New Jersey.

Benzmalecene, an hepatic cholesterol synthesis inhibitor (Huff and Gilfillan; Proc. Soc. Exper. Biol. & Med., November 1959), lowers blood cholesterol in dogs (Baer, Brooks, Russo, and Beyer; The Pharmacologist, Fall 1959). MK-135, an anion exchange resin that sequesters bile acids, lowers blood cholesterol in cockerels and in dogs (Tennent, Siegel, Zanetti, Kuron, Ott, and Wolf; Circulation 20, 969, 1959). The influence of the combination of these substances on plasma cholesterol of normocholesteremic cockerels and dogs is reported here. Feeding of benzmalecene to normocholesteremic cockerels did not lower their plasma cholesterol levels, but intramuscular injection of benzmalecene did do so. Similarly, in MK-135-fed birds, feeding of benzmalecene did not cause additional plasma cholesterol lowering, but injection of benzmalecene did do so. In dogs, both MK-135 and benzmalecene lowered plasma cholesterol level when fed in the diet, and had additive effect when fed in combination.

4 *Biochemistry*

FLUOROMETRY OF BLOOD PROGESTERONE. J. C. Touchstone and T. Murawec.* Dept. of Obst. and Gyn., Medical School, Univ. of Penna. Philadelphia, Pennsylvania.

Ether extracts of blood plasma were separated by chromatography on alumina columns. Prior to chromatography, partition between heptane and 70% methanol served to remove cholesterol and fats. After development with heptane and by gradually increasing the chloroform content of this solvent, progesterone was finally eluted with 30% chloroform in heptane. A new fluorometric method consisting of heating progesterone at 60° for 30 minutes in 2N potassium hydroxide in methanol before dissolving it in sulfuric acid at room temperature resulted in a 100 fold increase of fluorescence over that found with sulfuric acid alone. This reaction caused the appearance of a new peak at 380 mμ in the absorption spectrum of progesterone in sulfuric acid. Florescence was maximal at 490 mμ when excitation was carried out at 390 mμ. By this procedure the estrogens and alpha ketolic cortical steroids showed a decrease in fluorescence intensity. Progesterone values averaging 20 μg. per 100 ml. were found in blood of pregnant women at term.

5 *Pharmacology*

COMPARATIVE EFFECTS OF MK-135 AND NICOTINIC ACID ON CHOLESTEROL LEVELS OF HYPERCHOLESTEROLEMIC HUMANS. DeWitt Hendee Smith and Elizabeth Gaman (intr. by Edmund W.J. DeMaar), Arteriosclerosis Laboratory, New Jersey Neuro-Psychiatric Institute, Princeton, New Jersey.

The resin MK-135 is designed to combine with cholic acid in the gut so that its effect in promoting reabsorption of excreted billiary cholesterol is diminished. Tennent et al (Circ.20:969, 1959) in animals and Van Itallie et al (Circ. 20:981, 1959) in certain humans showed such cholesterol lowering. The present experiments are designed to compare the effect of this resin, nicotinic acid, and the combination. Four groups of 8 hypercholesterolemic institute patients matched for cholesterol level and medication were placed for a preliminary period on 3 grams of placebo tablets, then respectively for 4 weeks on divided daily doses of MK-135 resin 15 grams, nicotinic acid 3 grams, and on the combination; the fourth group was kept on placebo. 14% cholesterol lowering occurred with MK-135 and with nicotinic acid, and 29% with the combination; all diminutions were significant. From these experiments the resin and the nicotinic acid appear to have comparable effects in the dosage used, and if combined have effects that are additive.
(Supported in part by grants from the National Heart Institute.)

6 *Pathology*

THE EFFECT OF HIGH FAT DIET ON SERUM LIPIDS OF YOUNG HEALTHY KOREAN. K. T. Lee, K. S. Kim and Y. S. Han. (intr. by Robert M. O'Neal.) Kyungpook Univ. School of Med., Taegu, Korea.

It has been believed that the diets of Korean people are extremely low in fat content, and their serum cholesterol and lipid phosphorus levels, and the frequency of coronary heart disease, are also remarkably low. An attempt is made to investigate changes in serum lipid pattern induced by the high fat diet. 90 Korean young soldiers attached to the 8th U.S. Army stationed in Taegu who are fed high fat American diets, 25 U.S. soldiers of similar age and 35 U.S. officers are selected for this study. They are divided into 7 groups, on the basis of periods in which they were on the American diet. Their diets were analysed and their various serum lipid fractions (total and free cholesterol and esters, total esterified fatty acids, lipid phosphorus, and neutral fats), C/P ratios and total serum proteins were measured for statistical analysis and comparison. The most striking difference between the Korean and American diets is the fat content; 15% of the total caloric intake is derived from fat in the Korean diet whereas 42% in the American diet. All the serum fractions of the Korean soldiers, which were remarkably low initially, began to rise within 1 month of their high fat diets and most of them reached almost as high levels as those of American soldiers of similar age in 12 to 18 months. (Supported by PHS grant No. H-2672(C).)

7 *Pathology*

SERUM LIPIDS AND ELECTROPHORETIC PATTERNS OF PATIENTS RECEIVING MULTIPLE INTRAVENOUS INFUSIONS OF A TRIGLYCERIDE EMULSION. H. C. Meng, J. S. Kaley and J. L. Shapiro. (Intr. by W. J. Cheatham) Depts. Physiol. and Pathol., Vanderbilt, Nashville.

Adverse reaction has been observed in some patients receiving multiple daily infusions of a triglyceride emulsion. This study is an attempt to correlate increase in serum turbidity, elevation of serum lipids, changes in serum electrophoretic patterns of proteins, lipoproteins and glycoproteins and their roles in causing adverse reaction. Results from 9 patients receiving from 20-29 consecutive infusions were: 1) The degree of turbidity of serum is usually not proportionally correlated with total serum lipids determined by chemical methods. 2) In 3 patients who had mild reaction, neither serum turbidity nor serum total fatty acids was significantly elevated; no ill effects were observed in patients with increased serum turbidity. 3) Serum lipoproteins showed an increase in beta-fraction, but a decrease in alpha-fraction. The mobility of beta-fraction was increased. These changes occurred in patients with and without reaction. 4) A decrease in albumin glycoprotein and a marked increase in alpha-glycoprotein were observed during adverse reaction.

8 *Nutrition*

SEX DIFFERENCES IN PLASMA LIPIDS IN RATS. Ruth Okey and Elaine W. Lis*. Dept. of Nutrition & Home Economics, Univ. of Calif., Berkeley, Calif.

In groups of young female rats fed diets containing cholesterol in certain vegetable oils, approximately two-thirds had been observed to have serum cholesterols ranging from 125 to 200 mg% while values for males and the rest of the females were under 85 mg%. We attempted to find out if the high cholesterols were associated with any phase of the estrus cycle or with esterification of any specific fatty acids. Diets containing 10% fat (coconut or cottonseed oil) with and without cholesterol were fed to weanling rats of both sexes ad libitum and in amounts limited by limitation of time of access to food. After 7 weeks on diet, vaginal smears were taken and the rats sacrificed. High plasma cholesterols were not consistently associated with any phase of the cycle. When cholesterol was fed there was a larger proportion of high plasma cholesterols in the females denied continuous access to food than in the ad libitum fed females or in males of either group, and the percent of arachidonic acid in cholesterol ester was lowered. If linoleate was fed, the percentage of ester linoleate increased, otherwise, that of oleate. There was about 10% saturated acid in the cholesterol ester, 35 to 50% in the other plasma lipids. Data for gas-liquid chromatographic separation of lipid fractions are to be reported. (Supported in part by grant H-2152, PHS.)

9 *Nutrition*

SERUM LIPIDS AS RELATED TO PREVIOUS DIET. R. E. Bleiler, E. S. Yearick, N. S. Springer and H. Stevens (Intr. by M. A. Ohlson). Nutrition Dept., State Univ. Iowa.

Four basal blood samples, detailed dietary records and histories have been obtained from 10 each of young men, young women, young women 7 months pregnant, and women of 50 or more years of age. All subjects were well and within normal weight limits. Serum cholesterol, total lipids and phospholipids were within expected ranges for young men and women. Mean serum cholesterol was 315 mg. percent for both pregnant and older women and total lipids were 720 and 663 mg. percent, respectively, with 33 percent of phospholipids in each case. All diets were deemed adequate. Percentage of fat and protein calories in the diet have been correlated with blood lipids. None of the correlation coefficients were significant, though certain comparisons approached significance. However, the rank order of r in the various subject groups suggested that both age and sex influence the effect of diet on fasting blood lipids and that serum cholesterol and total lipids are subject to different regulatory controls.

10 *Nutrition*

EFFECT OF TRIPARANOL ON BAPN-INDUCED DISSECTING ANEURYSM AND PLASMA LIPIDS IN THE TURKEY. P. E. Waibel, R. E. Burger*, R. A. Bell*, J. H. Sautter*, I. E. Liener, and B. S. Pomeroy*. Institute of Agriculture, University of Minnesota, St. Paul.

Triparanol (formerly designated MER-29, Merrell Co., Cincinnati, Ohio) depresses the plasma cholesterol level in rats (Hlohm et al., Fed. Proc. 18: No. 1, 369, 1959). The occurrence of fatty infiltration in the aortic intimas of turkeys succumbing from naturally-occurring dissecting aneurysm (Carnaghan, R. B. A., Vet. Rec. 67: 568, 1959) raised the question of a possible relationship between plasma cholesterol and phospholipid levels and BAPN-induced dissecting aneurysm. The addition of 25-1600 mg of triparanol per kg of diet of turkey poults lowered the ratio of blood plasma cholesterol to phospholipid by usually decreasing cholesterol and increasing phospholipid. The incidence of BAPN-induced dissecting aneurysm was not lessened by triparanol. The 25 mg/kg level of triparanol caused some growth depression. The degree of growth depression was proportional to the logarithm of the dose. It is concluded that decreasing the cholesterol to phospholipid ratio of turkey blood plasma did not lessen the severity of BAPN-induced dissecting aneurysm. (Supported by grant H-4177 from National Heart Institute, Public Health Service)

11 *Nutrition*

NICOTINIC ACID AND SERUM CHOLESTEROL IN THE DOG. Francisco Grande and Donald S. Amatuzio*. Jay Phillips Research Lab. Mount Sinai Hosp. and U. of Minnesota, Minneapolis, Minnesota.

The effect of nicotinic acid (NA) on the hypercholesterolemia produced in dogs by ethanol was studied. 3 dogs (mean wt. 20.4 Kg) kept on a high fat diet (40% fat calories)and 1.65 g. ethanol/kg/day, when given 1.2 g NA daily for 2 weeks showed no cholesterol change. Another 6 dogs (mean wt. 17.5 kg) on the high fat diet, when given ethanol (1.65 g/kg/day) for 2 weeks had a mean cholesterol increase of 81 mg/100 ml (SE = 19). When NA (1.2 g/day) was added to the high fat diet, ethanol caused a mean cholesterol increase of 54 mg/100 ml (SE = 20). The mean difference between the responses (27 mg /100 ml, SE = 24) was not significant (p = 0.25). NA (1.2 g/day) given to dogs on high fat diet without ethanol failed to produce significant cholesterol changes in 5 dogs (mean wt. 17.8 kg) after 2 weeks, and in another 6 dogs (mean wt. 20.0 kg) after 3 weeks. It is concluded that nicotinic acid does not prevent the cholesterol increase produced in dogs by ethanol, and that it does not decrease cholesterol in dogs when given at doses higher than those which are effective in man.

153. LIPIDS–Sterols–Diet

1 *Biochemistry*

The Effect of Lactose-Containing Diets on Sterol Metabolism in the Rat. W.W. Wells, S.C. Anderson* and R. Quan-Ma*. Biochemistry Dept., Univ. of Pittsburgh, School of Medicine, Pittsburgh, Pa.

Previous observations in this laboratory have shown that cholesterol absorption in the rat and rabbit is enhanced by a lactose-containing diet indicated by measurement of serum and liver cholesterol concentration or cholesterol balance technique. We have confirmed these findings by the direct measurement of cholesterol-4-C^{14} collected from thoracic lymph duct cannulas of animals previously fed sucrose or 40% lactose diets and given 27.2 mg. of cholesterol-4-C^{14} (150,900 c.p.m.) in 1 ml. of cotton seed oil. The average of the first 24 hour recovery of radioactivity from the sucrose controls was 7.5% while the lactose-fed group averaged 18.9% recovery. We have studied the incorporation of injected Na acetate-2-C^{14} (5μC) into the digitonin precipitable sterol fraction of the livers of rats fed cholesterol-free diets. The diets contained the following additions at the expense of sucrose: 1) 40% lactose, 2) sucrose, 3) 20% galactose, 4) 40% starch, 5) 2% sulfa suxidine, terramycin mix. The average specific activities of the liver sterols from the above dietary groups were 6.7, 65.3, 76.3, 116.3, and 105.8 c.p.m./mg. respectively. A proposed mechanism for the effect of lactose on cholesterol absorption will be discussed.

154. LIPIDS–Sterols–Metabolism

1 *Biochemistry*

INTERMEDIATES IN A PATHWAY FROM LANOSTEROL TO CHOLESTEROL. A.A. Kandutsch and A.E. Russell*. Roscoe B. Jackson Mem. Lab., Bar Harbor, Me.

Sterols identified in a preputial gland tumor are lanosterol, 24,25-dihydrolanosterol, 4α-methyl-Δ^8-cholestenol, Δ^7-cholestenol, 7-dehydrocholesterol and cholesterol. Arranged in the above order, they suggest a possible sequence of steps in the conversion of lanosterol to cholesterol. In the present studies evidence that the suggested pathway does operate was provided by the kinetics of acetate incorporation into the sterols by preputial gland tumor slices and by metabolic conversions of the labeled sterols by cell-free homogenates of the tumor. The apparent presence of most or all of the above sterols in a variety of tissues suggests that the pathway is not unique to the preputial gland tumor. Further support for the existence of the pathway in other tissues was the conversion by cell-free homogenates of liver of 24,25-dihydrolanosterol, 4α-methyl-Δ^8-cholestenol, Δ^7-cholestenol, and 7-dehydrocholesterol to cholesterol in the following percentages: 18, 32, 67, and 74 respectively. (Supported by grants C-2758 and C-1329 from the National Cancer Institute, National Institutes of Health, United States Public Health Service.)

2 *Biochemistry*

EFFECT OF CASTRATION AND OF ESTROGENS ON OXIDATION OF CHOLESTEROL-26-C^{14} BY MALE RAT LIVER MITOCHONDRIA. D. Kritchevsky, M. W. Whitehouse & E. Staple. The Wistar Inst., & Dept. of Biochem., School of Med., Univ. of Penna., Phila. 4 Penna.

We have observed that female rat liver mitochondria consistently oxidized more cholesterol and pyruvate than did male rat liver mitochondria. To test the effect of "maleness" four groups of male rats were treated as follows: male control (M), castrated (MC), estrogenized (15γ estradiol benzoate /150 gm daily) (ME), and castrated-estrogenized (MEC). After two weeks of treatment oxidation experiments were begun, using cholesterol-26-C^{14} and octanoate-1-C^{14} as substrates. The $C^{14}O_2$ liberated (18 hr. incubation) was collected and assayed. Results corrected for the amount of nitrogen present in the mitochondrial preparations. Male rats oxidized less cholesterol than did castrated or femininized rats. Disparities in oxidation rates became larger as treatment progressed. % oxidation of cholesterol after 27 days of treatment was: M-7.2; MC-32.5; ME-35.6; and MEC-30.9. After 30 days on treatment serum cholesterol levels (mg %) were: M-61.0 ± 10.3; MC-80.3 ± 18.0; ME-79.4 ± 13.0; MEC-92.2 ± 17.0 Liver cholesterol levels (mg/100 gm) M-78.9 ± 10.6; MC-97.4 ± 10.1; ME-92.2 ± 4.7; and MEC-105.6 ± 5.5. (Grant H-3299, Natl. Insts. of Health.)

XUM

3 *Biochemistry*

IN VIVO METABOLISM OF MEVALONIC ACID-2-C^{14}. J. C. Elwood* and J. T. Van Bruggen. Dept. of Biochemistry, Univ. of Oregon Medical School, Portland, Oregon.

The early time course (0-4 hrs.) of cholesterol labeling, $C^{14}O_2$ evolution, C^{14} activity of blood and urine, and the formation of labeled acidic products were studied following the intraperitoneal injection of MVA-2-C^{14} to young male rats. It is concluded that cholesterol synthesis from MVA-2-C^{14} is complete within the first hour post injection. The subsequent changes in specific activity of the various tissues is considered to be due to transport. The half-life ($t_{\frac{1}{2}}$) values of excretion by the liver and accumulation by the gut are given. The liver is shown to have a very labile cholesterol component, $t_{\frac{1}{2}}$ = 1.7 hrs., and a slower component with $t_{\frac{1}{2}}$ = 5 hrs. The gut is suggested to be the recipient of the cholesterol from the liver presumably via the entero-hepatic circulation. The carcass fraction is shown to be a metabolically heterogeneous group of tissues with the kidneys containing 55-70% of the radioactivity that has been attributed to the carcass fraction. A time course study of the kidney provided information on the role of the kidney in cholesterogenesis. In agreement with the synthesis data, the C^{14} activity of blood and urine indicate very rapid clearance and excretion of radioactive materials. (Supported by grant no. A-2305 from the National Institutes of Health.)

4 *Biochemistry*

EFFECTS OF BILE ACIDS ON CHOLESTEROL METABOLISM IN THE MOUSE. W. T. Beher, G. D. Baker*, and W. L. Anthony*. Edsel B. Ford Institute for Medical Research, Henry Ford Hospital, Detroit.

In mice cholic acid caused increased liver cholesterol levels accompanied by decreased synthesis rates. On the other hand, hyodeoxycholic acid reduced liver cholesterol, and accelerated the synthesis rate. Observations were made on the effects of these two acids on the metabolism of cholesterol of exogenous origin and on the regression of accumulated tissue cholesterol. In the former study, mice were fed diets supplemented with bile acids, with cholesterol, or with a combination of these. In the regression study, after a tissue cholesterol build-up period, diets were supplemented with bile acids, and subsequent cholesterol regression rates determined. Cholic acid caused accumulation of liver cholesterol and decreased the rate of mobilization. Hyodeoxycholic acid prevented liver cholesterol accumulation, increased the rate of mobilization, and reversed the effects of cholic acid. It is concluded that hyodeoxycholic acid, acting as an antimetabolite of cholic acid, is useful in the control of mouse liver cholesterol levels.

5 *Biochemistry*

METABOLISM OF CHOLANIC-24-C^{14} ACID IN THE RAT. P. D. Ray,* R. L. Ratliff,* Lester W. Wells,* and S. A. Thayer. Dept. of Biochemistry, St. Louis Univ. Sch. of Med., St. Louis, Mo.

Cholanic-24-C^{14} acid was prepared from deoxycholic-24-C^{14} acid by chromic acid oxidation and subsequent Wolff-Kishner reduction. Approximately 0.7 mg. (sp. act. 2.55 x 10^6 d/m/mg.) of the acid as its sodium salt was administered intraperitoneally to rats with bile fistulas or ligated bile ducts. An average of 91% and 80% of the administered C^{14} was recovered in the bile and urine, respectively, within 96 hr. after administration. No C^{14} was detected in the expired air of the duct ligated animals. The pooled bile samples from each animal were autoclaved with 5% NaOH. The free acids were extracted and chromatographed on an aqueous acetic acid partition column. Of the chromatographed C^{14}, 13% appeared in the β-muricholic acid fractions and 20% in the α-muricholic acid fractions. Radioactive β-muricholic acid was identified by isotopic dilution, isolation and chromatography. An average of 43% of the chromatographed C^{14} obtained from urine of jaundiced rats after similar treatment was found in the β-muricholic acid fractions. These experiments demonstrate the ability of the rat to hydroxylate the bile acid skeleton at positions 3, 6 and 7.

6 *Biochemistry*

METABOLISM OF α-MURICHOLIC ACID IN THE RAT. George D. Cherayil,* Robert G. Bell,* and William H. Elliott. Dept. of Biochemistry, St. Louis Univ. Sch. of Med., St. Louis, Mo.

α-Muricholic acid-24-C^{14} (0.7 mg., 1.3 x 10^7 c.p.m.) was administered intragastrically as the sodium salt in aqueous solution to normal, bile fistula and surgically jaundiced rats. Most of the C^{14} (87-100%) was recovered in the urine of jaundiced rats over a 10 day period. After extraction with butanol and hydrolysis with 5% NaOH, the free acids were chromatographed on acetic acid partition columns. In three experiments, from 36 to 43% of the chromatographed C^{14} was found in the β-muricholic acid fractions; from 11 to 16% appeared in the α-muricholic fractions. Radioactive muricholic was isolated and characterized, thus extending the work of B. Samuelsson (Acta Chem. Scand. 13, 976 (1959)). In addition, radioactive chenodeoxycholic acid (3.5%), 6β-hyodeoxycholic acid (0.6%) as well as α-muricholic acid were identified by isotopic dilution.

7 *Biochemistry*

INFLUENCE OF DIETARY MINERALS IN BILE ACID PRODUCTION IN THE RAT. Mao-Wu Yin and James W. Ahlberg (intr. by Philip A. Katzman.) Dept. of Biochemistry, St. Louis Univ. Sch. of Med., St. Louis, Mo.

Previous reports from this laboratory state that rats raised on Purina chow produce in bile about 2.5, 0.26, and 0.23 mg./ml. of cholic, α- and β-muricholic acid, respectively. Animals on purified rations secrete similar amounts of cholic acid but about 0.05 mg./ml. of the latter acids, based on C^{14} dilution experiments. In studying possible causes of this difference, the salt mixture of our purified diet was supplemented with 0.1-1.0 p.p.m. of 16 minerals not customarily added. Maximal production of 0.29 and 0.125 mg./ml. of α- and β-muricholic acid, respectively, was observed. In other experiments the production of α-muricholic acid was increased by the addition of Mo and Cu to the diet. Mo alone decreased β-muricholic acid production in one experiment while the addition of Mo and Cu increased this acid above the basal level.

8 *Biochemistry*

THE METABOLISM OF CHENODEOXYCHOLIC ACID-24-C^{14} IN SURGICALLY JAUNDICED MICE. Vincent A. Ziboh* and S. L. Hsia, Dept. of Biochemistry, St. Louis Univ. Sch. of Med., St. Louis, Mo.

After intragastric administration of sodium chenodeoxycholate-24-C^{14} (0.5 ml., 9.45 x 10^5 c.p.m.) to each of a group of surgically jaundiced mice, about 46% of the C^{14} appeared in the urine over 11 days. After alkaline hydrolysis, the free urinary acids were subjected to partition chromatography. Distribution of C^{14} in the eluates indicated extensive metabolism; about 16% of the chromatographed C^{14} appeared in the β-muricholic acid fractions and 11% in the α-muricholic acid fractions. Both acids were identified by isotopic dilution, thus extending the observations of Danielsson and Kazuno (Acta Chem. Scand., 13, 1141 (1959)). No conversion to cholic acid could be detected, but two dihydroxycholanic acids, ursodeoxycholic acid (3α, 7β-diol) and 3α, 6β-dihydroxycholanic acid, were identified by isotopic dilution from fractions containing 7% of chromatographed C^{14} dihydroxycholanic acids.

9 *Biochemistry*

OXIDATION OF TRIHYDROXYCOPROSTANIC ACID-26-C^{14} TO $C^{14}O_2$ IN VITRO. Thomas Briggs* and Ezra Staple. School of Medicine, University of Pennsylvania, Philadelphia, Pennsylvania.

Trihydroxycoprostanic acid, found in the bile of some "primitive" vertebrates, may be an intermediate in the formation of bile acids from cholesterol in mammals. Carboxyl-labeled trihydroxycoprostanic acid was prepared biosynthetically, and its oxidation to $C^{14}O_2$ by washed rat liver mitochondria preparations was studied. Incubations were carried out at pH 8.5, and CO_2 was collected. If 1 mg. of unlabeled trihydroxycoprostanic acid was present in the incubation mixture (12 ml.) with cholesterol-26-C^{14}, the $C^{14}O_2$ evolved was depressed to 12% of the control incubation containing cholic acid and labeled cholesterol. Trihydroxycoprostanic acid-26-C^{14} (0.5 mg.) was oxidized to the extent of 45% by similar mitochondrial preparations. These experiments with C^{14} labeled substrate show that the enzymes capable of oxidizing trihydroxycoprostanic acid are located in the mitochondria. The oxidation proceeds rapidly, supporting the view that this acid may be an intermediate in the conversion of cholesterol to bile acids in the mammal.

10 *Biochemistry*

OBSERVATIONS ON BODY CHOLESTEROL METABOLISM IN MAN. Aram V. Chobanian and William Hollander (intr. by F.M. Sinex). Mass. Mem. Hosps. and Boston Univ. School of Med., Boston, Mass.

Tracer doses of C-14 cholesterol have been administered intravenously to 11 human subjects with and without hypercholesterolemia and the disappearance from the serum of cholesterol radioactivity has been studied over a 3 month period. Following a 4-5 week period when serum cholesterol specific activity decreases relatively rapidly, the fall in serum specific activity in each instance approaches an exponential rate. The apparent miscible pool of cholesterol as calculated from this linear slope of the specific activity curve has ranged from 0.16 to 0.31% body weight and the apparent half-time disappearance of cholesterol has varied between 42 and 61 days. No consistent relationship has been observed between the serum cholesterol and the miscible pool or turnover rate of cholesterol. These observations suggest that indices of body cholesterol miscible pool and turnover rate may be obtained in man from isotopic studies.

11 *Pathology*

CHOLESTEROL-4-C^{14} AND BILE ACIDS IN THE GUINEA PIG. Ludvik Peric-Golia* and Russell S. Jones. Dept. of Pathology, Univ. of Utah, College of Med., Salt Lake City, Utah.

The conversion of cholesterol-4-C^{14} to bile acids and the interconversion of the bile acids has been studied in the guinea pig with catheter passing from gall bladder over the back and into the proximally ligated common bile duct. Labelled cholesterol was injected intravenously and bile either sampled during enterohepatic cycling or collected in toto. By supplying an equal volume of bile for that removed, the guinea pigs survived several weeks. Bile collected in toto according to time intervals was given to different guinea pigs by catheter through the common bile duct into the duodenum. Bile from these animals was then collected during the following weeks. The sterols and bile acids were isolated and identified by column and paper chromatographic procedures and the percent incorporation of C^{14} determined. The enterohepatic cycling of bile acids continued for some weeks but labelled cholesterol rapidly diminished in the bile. All radioactivity was in the predominant taurine conjugated bile acids. Of these, cholic acid showed the highest relative labelling. After several days the only detectable bile acid was taurocholic acid. Of the various bile acids given as mixed samples by common bile duct catheter, taurine conjugated cholic acid showed the least loss in relative isotopic content during enterohepatic cycling.

12 *Pathology*

ENZYMATIC REDUCTION OF PREDNISOLONE TO CORTISOL. Edward L. Rongone (Intr. by Albert Segaloff) Alton Ochsner Medical Foundation, New Orleans, La.

Incubation of natural steroids containing a 3 keto-4,5-double bond in ring A with a commercial preparation of bovine blood albumin in the presence of TPN and isocitrate results in the loss of absorption at 238 mμ. Chemical changes to the steroid nucleus, if they have a proximity to Ring A, tend to affect the reducibility of the double bond in Ring A. The addition of a 2α methyl group to testosterone completely inhibits the loss of absorption at 238 mμ, whereas the addition of a 1,2 double bond to cortisol tends to cause a decrease in the rate of loss of absorption at 238 mμ. When 2α methyl testosterone and Prednisolone were incubated with 5% bovine blood albumin solutions containing 3% ethanol 2α methyl testosterone, prednisolone and cortisol were respectively isolated. The products were identified by melting points, mixed melting points, glass paper chromatography, and sulfuric acid spectra. (Supported by a grant from the Am. Can. Soc.)

155. LIPIDS–Sterols–Synthesis

1 *Physiology*

COUNTER CURRENT DISTRIBUTION AND ELECTROPHORESIS OF CRF, LYSINE VASOPRESSIN AND α-MSH. Roger Guillemin, Andrew V. Schally*, Richard N. Andersen* and John L. Long*. Dept. of Physiology, Baylor U. Coll. Med., Houston, Texas.

CRF concentrates (PSEBM 104, 777, 1958; Tex. Rep. Biol. & Med., March 1960) were subjected to 100 transfers by CCD using the 3 systems: 1.n-butanol,.1M p-toluene sulfonic acid; 2.n-but,.05M p-tol.sulf.ac.; 3.n-but,pyridine,.1% HOAc,5:3:11. In the 3 systems, LVP and CRF activity are widely separated (K^1=.52 vs ca 13.0; K^2=.38 vs ca 7.2; K^3=.57 vs 1.8). No CRF activity is demonstrable in the LVP peak (300 USPu/mgm). These data confirm once more our repeated findings that CRF is distinct from vasopressin. On the other hand, in the 3 systems, the peak of CRF activity corresponds to the peak of α-MSH ($1\text{-}2 \times 10^6$ u/mgm). Paper strip or zone electrophoresis, under the conditions used so far has failed to achieve a conclusive separation of CRF activity from α-MSH. On the basis of these data, it cannot be ascertained whether CRF and α-MSH are or are not the same molecule, an observation which might clarify the biological significance of α-MSH in mammals. The inherent adrenocorticotrophic activity of α-MSH (PSEBM 101,394, 1959) and possible biosynthesis of ACTH from α-MSH (Critchlow, Lipscomb, Guillemin, unpublished) complicate further the matter.

2 *Physiology*

INHIBITION OF CONVERSION OF 24-DEHYDROCHOLESTEROL TO CHOLESTEROL BY MER-29. J. Avigan, D. Steinberg, M. J. Thompson and E. Mosettig (intr. by R. S. Gordon). Natl. Heart Inst. and Natl. Inst. Arthritis and Metabolic Diseases, Bethesda, Md.

MER-29 (1-[p-(β-diethylaminoethoxy)-phenyl]-1-(p-tolyl)-2-(p-chlorophenyl) ethanol) strongly inhibits cholesterol biosynthesis and depresses serum and liver cholesterol levels (Elohm and McKenzie, Arch. Biochem. Biophys. 85, 245 (1959)). We found that in rats fed 10 mg. of the drug daily for 14 days or longer the digitonin-precipitable liver sterols showed a significantly lower melting point than pure cholesterol and no dibromide could be precipitated from these sterols. Chromatography of p-phenylazobenzoyl esters yielded two components in approximately equal amounts; one was identified as cholesterol (I), the second was found to be 24-dehydrocholesterol (II) (mixed melting point, infrared spectrum, analysis of brominated product). A rat treated with MER-29 was injected with 1-C^{14}-acetate, killed 4 hours later and the liver sterols isolated. Specific radioactivity of II was 44 times that of I. It is concluded that MER-29 blocks conversion of II to I and that II is an obligatory intermediate in cholesterol biosynthesis. II was found in significant amounts in the blood of patients treated with MER-29. The significance of this finding in evaluation of the drug as a therapeutic agent will be discussed.

3 *Biochemistry*

MEVALONIC KINASE IN RABBIT LIVER. Kehl Markley and Elizabeth Smallman (intr. by E. O. Keiles.) Natl. Insts. of Health, Bethesda, Md.

Mevalonic kinase has been purified 100-fold from an acetone powder of rabbit liver by ammonium sulfate fractionation, protamine sulfate precipitation, and DEAE-cellulose chromatography. This enzyme catalyzes the formation of phosphomevalonic acid and ADP from the biologically active isomer of mevalonic acid and ATP. The enzyme is free from myokinase and ATP-ase. Cysteine (or to a lesser extent other -SH compounds), phosphate, and Mg^{++} or Mn^{++} are required for activity. Guanosine, uridine, or inosine triphosphates cannot effectively replace adenosine triphosphate. The enzyme is inhibited by p-chloromercuribenzoate (83% inhibition at 10^{-4} M), but activity can be fully restored by incubation for an hour with an excess of cysteine or glutathione (3×10^{-2} M).

4 *Biochemistry*

PHOSPHORYLATION OF MEVALONIC ACID BY PLANT EXTRACTS. W. D. Loomis and J. Battaile (intr. by L. F. Remmert) Science Research Inst. and Dept. of Chemistry, Oregon State College, Corvallis, Ore.

Cell-free extracts from pumpkin seedlings form two phosphorylated derivatives of mevalonic acid(MVA) in the presence of MVA-2-C^{14}, ATP, and Mn^{++}. The derivatives appear to be identical to compounds formed by yeast enzymes (Tchen, J. Biol. Chem. 233: 1100, 1958; Chaykin et al, Proc. Nat. Acad. Sci. 44: 998, 1958), namely 5-phosphomevalonic acid (P-MVA) and 5-pyrophosphomevalonic acid (PP-MVA). R_f values of the derivatives in two different solvents agree with those reported for P-MVA and PP-MVA. In addition, the derivative which appears to be P-MVA is shown analytically to contain one mole of phosphate (not energy rich) per mole of MVA. This derivative is formed rapidly from MVA, while the presumed PP-MVA is formed more slowly. The latter can also be formed by the same extracts from P-MVA as a substrate, again in the presence of ATP. The requirement for a metal ion in the formation of P-MVA is met most effectively by Mn^{++}, but Co^{++}, Mg^{++}, and Fe^{++} are also active. Ca^{++} and Zn^{++} are inactive. ATP can be replaced by several other nucleoside triphosphates.

5 *Biochemistry*

STUDIES ON THE CONVERSION OF ZYMOSTEROL TO CHOLESTEROL G.J. Schroepfer, Jr. (intr. by W.D. Armstrong) Depts. of Physiological Chem. and Medicine, Univ. of Minnesota, Mpls.

In MER-29-treated rats the accumulation in liver of a sterol other than cholesterol has been noted. This sterol has been identified as desmosterol by Avigan and Steinberg (unpubl.) on material isolated from pig liver. This identification has been confirmed by Frantz and Mobberley (unpubl.) on the sterol isolated from the livers of MER-29-treated rats. In liver homogenates of treated rats the latter workers found the conversion of lathosterol to cholesterol to be normal. In the present study zymosterol-C^{14} was isolated from yeast incubated with acetate-1-C^{14} by a modification of the method of Johnston and Bloch (J.A.C.S. 79, 1145, 1957). When given intraportally, 52 and 2.3 per cent of the radioactivity recovered from the livers of normal and MER-29-treated rats, respectively, were associated chromatographically with cholesterol. These findings are compatible with a block in the reduction of the 24,25-double bond by MER-29. In both normal and treated rats several unidentified compounds were detected after giving zymosterol. In an attempt to study the shift of the nuclear double bond from the 8,9- to the 5,6-position, Δ^6-cholestenol-4-C^{14} was prepared and administered intraportally to normal rats. No conversion to cholesterol was detected.

6 *Biochemistry*

CONVERSION OF 4 α-METHYL-Δ^7 CHOLESTEN-3 β-OL-H^3 TO CHOLESTEROL BY RAT LIVER. Ivan D. Frantz, Jr., Marita Ener*, and Mary L. Mobberley*. Depts. of Medicine and Physiological Chemistry, Medical School, Univ. of Minnesota, Minneapolis, Minn.

Methostenol was prepared according to Neiderhiser and Wells (Arch. Biochem. Biophys. 81, 300, 1959), tritium being introduced at the 5 and 6 positions in the hydrogenation of 7-dehydrocholesterol to Δ^7-cholesten-3 β-ol. 1.2 mg. of methostenol-H^3 (140,000 c.p.m.) was dissolved in 0.1 ml. of absolute ethanol, and 0.8 ml. of physiological saline was added. The suspension was injected into the portal vein of a 200 gram female rat. Twenty hours later the liver was removed, and sterols were recovered by extraction of the unsaponifiable fraction and purification by way of the digitonide (80,000 c.p.m. recovered) and dibromide. Chromatography showed that almost all of the remaining radioactivity was present as cholesterol, which contained 3720 c.p.m. When a rat liver homogenate was incubated with methostenol-H^3 (22.5 μg., 2633 c.p.m., 22.5 ml. of incubation fluid), 11 per cent of the recovered radioactivity was associated with cholesterol. No conversion occurred if magnesium, DPN, and nicotinamide were omitted, although the same preparation converted lathosterol-H^3 to cholesterol in good yield without the three additives.

7 *Biochemistry*

EFFECT OF MEVALONATE ANALOGS ON THE BIOSYNTHESIS OF CHOLESTEROL IN VITRO. **E. Schiffmann and H. Weiss (intr. by Elwood Titus.) Natl. Heart Inst., Bethesda, Md.**

Mentzer et al. (Compt. rend. 242: 943, 1956) have reported that the feeding of Δ^3-3-methyl pentenoic acid reduces the level of cholesterol in the livers of rats while the Δ^2-isomer does not. Intramuscular administration of the Δ^3-compound to pigeons fed an atherosclerogenic diet both removed and prevented atherosclerotic changes (Supniewski et al. Bull. acad. polon. sci. 7: 199, 1959). These two compounds and a third, 3-methyl, 3-hydroxy pentanoic acid, which all resemble mevalonic acid, were synthesized and tested for their efficacies in reducing the incorporation of potassium mevalonate-2-C^{14} into cholesterol in the Bucher rat liver system. The order of efficacies is as follows: hydroxy acid > Δ^2-acid > Δ^3-acid; half maximal inhibition of cholesterol formation occurred at concentrations of 5 μM/ml, 15 μM/ml, and 22 μM/ml respectively. Pre-incubation of the hydroxy acid in the standard test system did not change the amount of mevalonate incorporated into cholesterol. The results indicate that the hydroxy acid is not a diluent i.e., is not converted to cholesterol via the mevalonate pathway, but is an inhibitor of sterol formation from mevalonate.

156. LYMPHATIC SYSTEM

1 *Physiology*

MAMMARY LYMPH FLOW AND COMPOSITION IN LACTATING GOATS. Monica Reynolds, Dept. of Physiology, School of Veterinary Med., Univ. of Penna., Phila., Penna.

Supramammary lymph ducts of goats were cannulated surgically with polyethelene tubing. The tube was brought to the outside through the posterior surface of the mammary gland from where it dripped lymph steadily for several weeks, affording an opportunity to make flow measurements and to analyse lymph composition. Although there was variation in lymph flow between individual goats and a gradual 2 to 3-fold increase throughout the average two week period of observation, the mean flow on the fourth day after surgery was 17 ml/hr. in 7 goats. There was also considerable variation in flow in the course of any one day. During milking itself lymph flow increased an average of 5 times the control. Concentrations of protein, sugars, NPN and electrolytes were measured in mammary lymph and venous serum. Protein concentration in the former ranged from 3 to 4.5 gms.% on the fourth day, while serum protein averaged 7.0 gms.%. Except for this difference, mammary lymph is similar to serum allowing for Gibbs-Donnan equilibrium adjustments. There was no significant difference between lymph from lactating and from non-lactating goats. (Supported by Research Grant H-4121 from the U.S. Public Health Service)

2 *Pathology*

OBSERVATIONS ON THE FATE OF THE LYMPHOCYTE, William J. Beckfield, M. Hirata*, Joseph Seifter and William B. Ehrich. University of Pennsylvania Graduate School of Medicine and Wyeth Institute for Medical Research, Philadelphia, Pa.

Living cells of rat thoracic duct lymph were injected into rat granuloma pouches prepared from a few minutes to several days earlier. The lymphocytes disappeared from the pouches at various speeds depending on the age of the pouches. The mode of their disappearance was elucidated by means of labeling in sections.

3 *Pathology*

INFLUENCE OF SUPRAVALVULAR MITRAL STENOSIS ON RATE OF FLOW AND CHEMICAL COMPOSITION OF THE RIGHT THORACIC DUCT IN DOGS. H. Namiki* and W. E. Jaques. Path. Dept., Univ. of Oklahoma Medical Center, Oklahoma City, Oklahoma.

The right thoracic duct was successfully cannulated in 18 dogs. Nine of these animals served as controls. The average lymph flow in the controls was 0.10 ml./kg./hr., the protein content 5.05 gm.%, chlorides 117 mEq/l, sodium 150 mEq/l and potassium 3.7 mEq/l. There was a marked variation in the termination of the right thoracic duct. Dogs with a supravalvular mitral stenosis showed an increase in lymph flow to 0.24 ml./kg./hr., a protein content of 7.07 gm.%, and no appreciable change in electrolyte content. Following acute ligation of the mitral valve for one hour, there was a further increase in rate of lymph flow and a slight increase in potassium content. The greatest increase in lymph flow closely paralleled an increase in pulmonary artery pressure. Histologic preparations showed pulmonary edema and marked edema of the sinuses of the hilar and tracheobronchial lymph nodes. It would appear that mitral stenosis is associated with an increased flow of lymph and a slight change in chemical composition. (Supported by a grant from Natl. Insts. of Health, #H-3235 C2.)

157. MICROORGANISMS

1 *Biochemistry*

NITROGEN FIXATION IN CELL-FREE EXTRACTS OF CLOSTRIDIUM PASTEURIANUM. I. PREPARATION OF EXTRACTS. J. E. Carnahan and J. E. Castle (intr. by Hans R. Rosenberg). Central Research Dept., E. I. du Pont de Nemours & Co., Inc., Wilmington, Del.

Reproducible nitrogen-fixing activity in cell-free extracts of a bacterium has been obtained for the first time. The extracts can be prepared from C. pasteurianum with either the Hughes press or by autolysis of dried cells. Sodium pyruvate must be added to obtain fixation. Activity assayed by the N_2^{15} method has consistently resulted in enrichments ranging 0.10-0.60 atom % excess N^{15}. That the active extracts are free of intact cells has been demonstrated by microscopic examination and centrifugation. Activity is not sedimented from solution during two hours at 144,000 x g. Except for α-ketobutyrate which produces weak activity, no other substance tested, including H_2, has been able to replace pyruvate in the extracts.

2 *Biochemistry*

NITROGEN FIXATION IN CELL-FREE EXTRACTS OF CLOSTRIDIUM PASTEURIANUM. II. MECHANISM OF FIXATION. Leonard E. Mortenson (intr. by T. R. Wood) Central Research Dept., E. I. du Pont de Nemours & Co., Inc., Wilmington, Del.

Time course experiments in which cell-free extracts of C. pasteurianum were fixing N_2^{15} showed that the label went initially to $N^{15}H_3$ and then to amino acids. In a 2-hr. experiment, 21 atom % excess N^{15} was found in glutamic acid isolated from a reaction mixture exposed to 60 atom % N_2^{15}. The bulk of the N_2^{15} fixed could be trapped as ammonia by adding a pool of ammonia to prevent by dilution the incorporation of $N^{15}H_3$ into amino acids. Addition of α-ketoglutarate favored glutamic acid production but did not increase fixation, indicating NH_3 acceptors were not limiting. Metabolism of pyruvate was essential for N-fixation since little fixation occurred until most of the pyruvate was consumed. Thus, a product of pyruvate metabolism (reducing power) is required for N-fixation.

3 *Biochemistry*

NITROGEN FIXATION IN CELL-FREE EXTRACTS OF CLOSTRIDIUM PASTEURIANUM. III. NITROGEN INDUCED DIFFERENCE ABSORPTION SPECTRA OF CELL-FREE EXTRACTS. Howard F. Mower (intr. by C. G. Niemann). Central Research Dept., E. I. du Pont de Nemours & Co., Inc., Wilmington, Del.

Cell-free extracts of C. pasteurianum show a spectral response when exposed to N_2. Extracts are first exposed to H_2. After equilibration, a portion is transferred anaerobically to a U.V. absorption cell. The atmosphere of the remaining portion is replaced by N_2. After equilibration, transfer is made to a second absorption cell. A difference is found between the absorption spectra of the two samples of 0.1 to 0.8 O.D. units with peak at 300 mμ. If argon is substituted for N_2, no response is obtained. O_2 induced differences are distinct from those of N_2. At concentrations less than 10 mg. protein/ml., peak position is concentration dependent.

4 *Biochemistry*

THE INCORPORATION OF THE NITROGEN ATOMS OF ADENINE INTO RIBOFLAVIN. Walter S. McNutt. Tufts University School of Medicine, Boston, Massachusetts.

The addition of U-N^{15}-adenine to Eremothecium ashbyii, cultured in a medium containing N^{14}-asparagine, resulted in extensive labeling of riboflavin. After dilution with carrier, the riboflavin had 0.133 atom % excess N^{15}. The chemical degradation products and the atom % excess N^{15} in each were as follows: Sodium 1,2-dihydro-1-D-ribityl-2-keto-6,7-dimethyl-3-quinoxaline carboxylate·H_2O(0.140); Dixanthydryl urea(0.123); 2-Amino-6,7-dimethyl-3-quinoxaline carboxylic acid(0.113). The interpretation of this experiment is that the biochemical intermediate between adenine and riboflavin retains not only the N atoms present initially in the pyrimidine ring of adenine but the N atoms which were present in the imidazole ring as well. These results suggest that in this organism the N atoms in the pyrazine ring of riboflavin do not arise from 1,2-dimethyl-4,5-diaminobenzene as proposed for other microorganisms (D.W. Woolley, The Strategy of Chemotherapy, Cambridge University Press, p.158, 1958). A similar experiment has been carried out making use of xanthine labeled with both C^{14} and N^{15}. I would like to thank Dr. Gerald O. Dudek of Harvard University for instructing me in the use of the mass spectrometer.

5 *Biochemistry*

ORIGIN OF POLYYNES IN POLYPORUS ANTHRACOPHILUS. J. D. Bu'Lock & D. C. Allport (intr. by E. Oginsky). The University, Manchester, England.

P. anthracophilus produces a mixture of polyyne substances of varying make-up (Bu'Lock, Jones, & Turner, J. Chem. Soc., 1957, p. 1607) and also triterpenoids, mainly eburicoic acid (Robertson et al., ibid, 1951, p. 2346). Both incorporate added (1-^{14}C) acetate; when the main polyyne $MeCH{:}CH(C{\equiv}C)_2\ CH{:}CH.CO_2Me$ is then isolated and degraded, $C_{(1)}$ and $C_{(9)}$ each have 1/5, and $C_{(10)}$ none, of the total activity, i.e. the chain is alternately labelled as in other polyynes (Bu'Lock & Gregory, Biochem. J. 72:322, 1959) and analogously to fatty acids. Incorporation into polyynes is reduced later in fermentation as breakdown and resynthesis occur, but that into triterpenes is relatively stable. The ratio polyynes/triterpenes in different strains varies, one appearing at the expense of the other, as with fat vs. isophrenoid synthesis in some other systems. As the last intermediate common to fatty acid and isoprenoid synthesis is probably acetoacetyl-CoA, the situation in P. anthracophilus suggests this is also a polyyne precursor, thus extending the analogy between polyyne and fatty acid synthesis.

6 *Biochemistry*

THE REDUCTION OF SULFATE TO SULFITE BY SOLUBLE ENZYMES OF DESULFOVIBRIO DESULFURICANS. H. D. Peck (intr. by M. I. Dolin.) Biol. Div., Oak Ridge Nat. Lab., Oak Ridge, Tenn.

Sulfate functions in the anaerobic respiration of both heterotrophically and autotrophically grown cells of Desulfovibrio desulfuricans as a terminal electron acceptor and is reduced to hydrogen sulfide. In cell-free extracts, ATP is required for the reduction of sulfate to sulfite or hydrogen sulfide with molecular hydrogen. In the presence of ATP and $S^{35}O_4^{--}$, one labeled nucleotide is formed and has been identified as adenosine-5'-phosphosulfate (APS). Heat-treated extracts are unable to reduce sulfate when supplemented with ATP but can reduce APS as indicated by the formation of acid-volatile S^{35}. 3'-Phosphoadenosine-5'-phosphosulfate is neither hydrolyzed nor reduced by these extracts. The ability to reduce sulfate when supplemented with ATP can be restored to heated extracts by adding purified yeast sulfurylase. The enzyme forming APS in extracts of D. desulfuricans has been separated by electrophoresis on Geon from pyrophosphatase and the enzyme reducing APS. It requires Mg^{++} for activity, catalyzes a sulfate-dependent ATP-pyrophosphate exchange, and appears similar to yeast sulfurylase. The products of APS reduction have been identified as AMP and sulfite. This enzyme has been named APS reductase and its assay, purification and properties will be discussed.

7 *Biochemistry*

PARTIAL CHEMICAL DEGRADATION OF THE CELL WALLS OF LACTOBACILLUS PLANTARUM AND STREPTOCOCCUS FAECALIS. Miyoshi Ikawa and Esmond E. Snell. Dept. of Biochemistry, Univ. of California, Berkeley, California.

Cell walls of L. plantarum or S. faecalis were extracted with 5% trichloracetic acid (TCA) at 90° or with 0.1N NaOH at 25°. The TCA-soluble fraction contains all of the reducing sugars and polyols together with D-alanine and most of the P; the insoluble residue contains most of the amino acid and amino sugar components of the wall. Both NaOH-soluble and insoluble fractions contain amino acids, aminosugars and non-nitrogenous wall components, but acidification of the soluble fraction from S. faecalis with acetic acid yields a precipitate low in aminosugars, high in the "trace" amino acids, and lacking both the reducing sugars and polyols of the wall. NaOH extraction of the TCA-insoluble fraction gives a similar result. These results indicate presence of at least two polypeptide components of the cell wall. TCA extraction of the NaOH-insoluble residue separates the amino acids from the non-nitrogenous sugars thus suggesting presence of more than one polysaccharide moiety. These results illustrate the complexity of the wall structure and provide some information on the modes of linkage of the various wall components. (Supported in part by a grant from the National Institute of Allergy and Infectious Diseases, U.S. Public Health Service.)

8 *Biochemistry*

CORRELATION OF LYSOZYME RESISTANCE TO CAROTENOGENESIS IN MICROCOCCUS LYSODEIKTICUS. A.L.N. Prasad* and Gerald Litwack. Dept. Ag.Biochemistry, Rutgers, The State Univ., New Brunswick, N.J.

Cells of lysozyme (L) sensitive strains of M.lysodeikticus are yellow in color; however, the organism, resistant to L, appears colorless. This phenomenon was examined quantitatively by determination of total carotenoid content and L lysability of normal and resistant strains. A strain of M.lysodeikticus, completely resistant to L,was obtained by successive subculture of the normal strain in liquid media containing increasing levels of L. After harvesting and washing the cells, carotenoids were extracted with 50 times the cellular wet weight of boiling methanol for 5 min. The absorption spectra of the clear, filtered methanolic extracts indicated the carotenoid concentration. Enzymic lysability was measured photometrically by the method of Litwack. The results demonstrate that M.lysodeikticus cells, completely resistant to L, contain no carotenoids under the conditions employed. Partially resistant cells are partially depleted of carotenoids. These findings suggest a relationship between L sensitivity and carotenogenesis. Further confirmation of this relationship is found when normal cells are made resistant via transformation with specific DNA (Chakrabarti and Litwack, unpublished). Partial characterization of 4 of the carotenoids from normal M.lysodeikticus will also be presented. (Supported by a grant, G-6459, from the National Science Foundation.)

9 *Biochemistry*

BIOSYNTHESIS OF PENICILLIC ACID. John Keil (intr. by M. Fuld Conderman), Dept. of Biochem. and Nutr., Univ. of Pittsburgh, Pittsburgh, Pa.

Degradations were carried out on labeled penicillic acid, $C^6H_2{=}C^5(\text{-}C^7H_3)\text{-}C^4O\text{-}C^3(\text{-}OC^8H_3){=}C^2H\text{-}C^1O_2H$, obtained from Penicillium cyclopium. Phenylhydrazine in acetic acid (Birkinshaw et al, Biochem. J., 30, 394 (1936)) gave a phenylpyrazoline phenylhydrazone (C 2-7), CO_2 (C 1) and methanol (C 8). Oxidation to the phenylpyrazole, followed by hypoiodite treatment gave iodoform (C 2) and a pyrazolemonocarboxylic acid; decarboxylation of the latter gave CO_2 (C 3). Kuhn-Roth oxidations on penicillic acid dibromide and the phenylhydrazone gave respectively acetic acid (C 5 + 7) and acetic acid (mixture of C 5 + 7 and 2 + 3), both further degraded by Schmidt reactions. Ozonolysis of penicillic acid gave formaldehyde (C 6). Penicillic acid obtained from L-methionine ($CH_3\text{-}C^{14}$) or sodium formate-C^{14} was labeled only in C 8. Although tartrate is required for penicillic acid synthesis, no incorporation of DL-tartrate-4-C^{14} occurred. With acetate-1-C^{14}, activity was found in C 1, 3 and 5; C 1 contained twice as much activity as C 3 or 5. With acetate-2-C^{14}, D-glucose-1- and 6-C^{14}, C 2, 4 and 6 were labeled; C 4 contained twice the activity of C 2 or 6. The primary biosynthetic pathway involves head to tail linkage of acetate, but contrary to Birch et al (J. Chem. Soc., 4582 (1958)) C 7 is not derived from acetate.

10 *Biochemistry*

TROPOLONE BIOSYNTHESIS - STIPITATONIC AND STIPITATIC ACIDS. Clara P. Thiessen * and Ronald Bentley. Dept. of Biochem. and Nutr., Univ. of Pittsburgh, Pittsburgh, Pa.

To study tropolone biosynthesis in molds, methods were developed for isolation and assay of stipitatonic and stipitatic acids. Stipitatonic acid was determined by measuring the fluorescence peak at 500 mμ (pH 2.5);stipitatic acid by difference from UV absorbancy values at 275 mμ (pH 12.5). A precursor role for stipitatonic acid was shown by the relative amounts of the acids in intact cultures of Penicillium stipitatum. With glucose or fructose grown cultures, stipitatonic values (all as mg/ml) were maximal (2.25) at 16-18 days, declining to 1.5 at 32 days. Stipitatic levels rose more slowly but eventually surpassed the stipitatonic peak at 2.75. On maltose, the stipitatonic peak was slightly higher (2.5),was maintained from 16-23 days, and was still 2.0 at 32 days; the final stipitatic level was 2.1. On sucrose, the stipitatonic peak was only 2.0 at 14 days; the final stipitatic level was higher (4.0) and the stipitatonic level lower (1.0) than with the other substrates. The precursor relationship was confirmed by extraction of a stipitatonic acid decarboxylase (Bentley and Thiessen, Nature, 184, 552 (1959)) which yielded stoichiometric amounts of stipitatic acid and CO_2. The enzyme was purified 35-fold by ammonium sulfate fractionation and by adsorption on and elution from calcium phosphate gel.

11 *Biochemistry*

NITROGEN FIXATION BY CELL-FREE PREPARATIONS FROM BLUE-GREEN ALGAE AND CLOSTRIDIA. K. C. Schneider*, R. N. Singh*, Clive Bradbeer* and R. H. Burris. Dept. of Biochemistry, Univ. of Wisconsin, Madison.

Blue-green algae disrupted by sonic oscillation and passed through millipore filters yielded cell-free preparations which fixed N_2. Small but readily detectable fixation was obtained with preparations from Nostoc muscorum, Anabaena cylindrica and Calothrix parietina, but Mastigocladus laminosus gave very active extracts. Nitrogen fixation was not confined to large particles, for the supernates from broken cells of M. laminosus centrifuged at 20,000 x g for 10 minutes or 144,000 x g for 60 minutes fixed N_2. The observation of Carnahan et al. has been confirmed that cell-free preparations of Clostridium pasteurianum supplied sodium pyruvate fix N_2.

12 *Biochemistry*

SEROLOGICAL RELATIONSHIPS OF AZOTOBACTER. Orville Wyss and Vera Imogene Russell.* Department of Bacteriology, University of Texas, Austin, Texas.

Antisera prepared against representatives of the various strains of Azotobacter were used to determine species relationships within the genus. Cross reactions confirm evidence obtained with bacteriophages that Azotobacter vinelandii strains are not properly classified as substrains of Azotobacter agilis. A study of cross reactions with other microorganisms supports the view suggested by morphological and physiological aspects that the azotobacter are more closely related to the blue-green algae than to the true bacteria. Agglutination, complement fixation and antibody adsorption show common antigens shared by a number of Azotobacter strains and the blue-green alga Anacystis nidulans. The most significant titers were demonstrated with cyst forming Azotobacter strains that produced large amounts of a flourescent pteridine pigment that is also found in large amounts in blue-green algae.

13 *Biochemistry*

CHANGES IN CHEMICAL COMPOSITION AND CELL SIZE ACCOMPANYING CESSATION OF MULTIPLICATION IN TETRAHYMENA PYRIFORMIS Y. W. J. Wingo and Wynelle D. Thompson*. Biochemistry Department, University of Alabama Medical Center, Birmingham.

Aerated axenic mass cultures of T. pyriformis Y were grown to densities of 6 to 10 x 10^5 cells/ml. in a medium similar to that of Hols et al. (J. Protozool., 6, 150, 1959). Homogenates of cells from the logarithmic phase were assayed for total N (as a basis of reference), for protein, and for four enzymatic activities, as were also homogenates of cells from the stationary phases of the same cultures. Protein and DPNH-oxidase activities did not differ significantly in the two states. Diaphorase and cytochrome-C reductase activities of multiplying cells were significantly higher, but the corresponding glutamic-oxaloacetic transaminase activities were much lower, than those of cells from the stationary phase. Both lengths and diameters of cells in the stationary phase were significantly greater than corresponding dimensions of cells from multiplying cultures. Thus significant changes in cell size and in the activities of at least some enzymes occur in close association with the end of cellular multiplication of T. pyriformis Y. (With the assistance of a grant from the Committee on Research, Council on Pharmacy and Chemistry, American Medical Association).

14 *Pathology*

A HEMIN-REQUIRING PLASMODIAL SLIME MOLD. Jacqueline Kelley*, J. W. Daniel*, and H. P. Rusch. McArdle Mem. Lab., U. Wis. Med. School, Madison, Wisconsin.

A growth factor required for the pure culture of Physarum polycephalum and previously found in chick embryo extract and several fractions of calf blood (Fed. Proc. 15: 513, 1956) can now be replaced by crystalline hemin. The cell yields and growth rate are the same on a natural medium containing either hemin or embryo extract. Cultures can be grown interchangeably on either factor without developing a marked adaptation to either one. On a synthetic medium containing the optimum hemin concentration, the mold proliferates more rapidly than in one containing embryo extract. Cytochrome-C and crystalline bovine hemoglobin have approximately five and ten fold the activity respectively, of hemin per mole of hemin; heat denatured hemoglobin is also active. Crystalline catalase and horseradish peroxidase are active only after mild heat denaturation. δ-Amino levulinic acid, protoporphyrin IX, a number of other prophyrins, ferriporphyrins, and bile pigments are inactive as embryo extract replacements when tested alone and are non-inhibitory in the presence of hemin. Several characteristics of a major growth promoting fraction of some embryo extract preparations suggest that the growth-active compounds identified so far account for only a portion of the whole extract activity.

15 *Immunology*

ELECTRON MICROSCOPY OF COCCIDOIDES IMMITIS. John O. Erickson and Abraham M. Breslau. Gen. Med. Res. and Radioisotope Service, V. A. Hosp., and Depts. of Radiology and Pathology, UCLA Med. Center, Los Angeles.

Organisms were grown in vitro in modified Roesseler's medium under CO_2 tension in special culture vessels at 37°C. All specimens for thin sectioning were fixed in cold OsO_4 and embedded in methacrylate. Isolated cell wall preparations were studied as shadowed whole mounts. Both filaments and spherules grown in vitro were examined in thin section, as was lung tissue from infected mice. Many of the structural features reported by O'Hern and Henry (J. Bact. 72,636 (1956) are confirmed in in vivo organisms and have been shown to also exist in in vitro organisms. The outstanding difference to date is the absence of the outer lipid layer always seen in vivo. The fibrillar nature of the spherule wall is demonstrated both in thin section and shadowed specimens of whole wall preparations. Preliminary experiments indicate that proteolytic enzymes remove matrix material from the spherule wall. (Aided by grants from U. S. Public Health Service and California Tuberculosis and Health Association.)

16 *Immunology*

INHIBITION OF THE CHAIN SPLITTING MECHANISM OF GROUP A STREPTOCOCCI BY ANTIBODY TO M PROTEIN. R.D.Ekstedt* and G. H.Stollerman. Northwestern Univ.Med.School, Chicago, Ill.

Virulent group A streptococci grow in short chains in serum-enriched media. The same strains lengthen markedly in plain Todd-Hewitt broth, and at suboptimum pH grow still longer. Whereas encapsulated, M-rich strains tend to form the shortest chains, neither M protein nor capsules are essential to chain shortening. Variants lacking either can form short chains. Contrariwise, only M-rich strains lengthen in homologous antiserum. All strains rich in M protein which have been studied form long chains providing anti-M antibody is present in excess. Minute amounts of M protein (less than 0.1 mcg.) can be detected accurately by specific inhibition of the long chain reaction.

When long chains are formed in antiserum they break up rapidly if an excess of soluble homologous M protein is added to the medium. This shortening reaction is temperature and pH dependent and is blocked by preliminary exposure of the long chains to killing doses of penicillin, $HgCl_2$, or heat. It is postulated that the union of M-anti-M in the cell wall inhibits a chain splitting enzyme which is synthesized by group A streptococci in protein enriched media.

XUM

17 *Immunology*

THE MULLER PHENOMENON - SATELLITE PROTEOLYSIS OF STAPHYLOCOCCUS AUREUS. Paul G. Quie* and Lewis W. Wannamaker, Univ. of Minn. Med. School, Minneapolis 14, Minnesota.

The Muller phenomenon is characterized by multiple discrete satellite areas of clearing around colonies of staphylococci on agar containing serum plus red cells or a suitable protein (e.g. hemoglobin) substrate (Elek, J. Hyg., 51:125, 1953). In the studies reported here, the staphylococcal factor was identified in the supernate of broth cultures and the phenomenon produced in a sterile cell-free system. Concentrates of the active supernate were subjected to starch zone electrophoresis, partially separating the Muller factor from other extracellular products. Staphylocoagulase and staphylokinase were found in the same zone on the starch block as the Muller factor. The serum Muller factor was found to have several properties similar to plasminogen. It is heat labile, is found in euglobulin, is inhibited by soybean trypsin inhibitor, and is activated by a bacterial filtrate. There are important differences, however: The pattern of lysis suggests that the serum factor is particulate or adsorbed on something particulate in serum; lytic action proceeds slowly; streptokinase-producing strains of streptococci fail to produce the phenomenon. An inhibitor of the Muller phenomenon, which migrates with gamma globulin on electrophoresis, has been identified in the sera of most human adults and in the sera of rabbits immunized with concentrates of staphylococcal culture supernates. Sera from immunized rabbits not only failed to support the phenomenon in the cell-free system but also inhibited an active system to a dilution of 1:32. Paradoxically, the sera of patients with serious chronic staphylococcal infections often lacked this inhibitor.

18 *Immunology*

OBSERVATIONS BY PHASE MICROSCOPY OF THE RESPONSE OF INTRA- AND EXTRACELLULAR SALMONELLA TYPHOSA TO ANTIBIOTIC THERAPY. H. E. Hopps, J. L. Showacre* and J. E. Smadel, Nat'l Inst. of Allergy and Infectious Diseases, NIH, Bethesda, Md.

The long periods of antibiotic treatment required to rid tissue culture cells of S. typhosa compared with rapid death of the organisms when exposed to these antibiotics in medium without cells led to direct observations of the effects of antibiotics on typhoid bacilli growing in L cells. Streptomycin (20µg/ml) inhibited intra- and extracellular bacterial multiplication within an hour. However, occasional L cells contained a few multiplying organisms in addition to many non-dividing bacilli. Resistance studies revealed that the seed inocula contained sufficient numbers of spontaneously resistant organisms to account for the few which multiplied intracellularly. Synnematin(20u/ml) or penicillin(100u/ml), antibiotics of similar structure, inhibited multiplication both intra- and extracellularly within minutes. Spheroplasts formed rapidly from most of the extracellular organisms but were seen only occasionally intracellularly; those in the latter position may have been phagocytized. Chloramphenicol (10µg/ml) promptly inhibited bacterial multiplication intra- and extracellularly; however, organisms outside the cells increased in size while those within were unchanged.

19 *Immunology*

IN VITRO STUDIES OF INTERACTION BETWEEN MOUSE PERITONEAL MACROPHAGES AND STRAINS OF SALMONELLA AND E. COLI. C.R. Jenkin*, D. Palmer* and B. Benacerraf. Depts. Path. and Med., New York Univ., New York.

A study has been made of the uptake and survival of different strains of Salmonella and E. coli in mouse peritoneal macrophages. Macrophages were suspended in 199 medium in Porter flasks, containing a flying coverslip; bacterial suspensions were added in a ratio of approximately 1:100 cells. After one hour incubation, the supernatant was removed and the coverslips incubated in fresh 199 for various periods of time. The uptake was calculated from the difference in bacterial concentration between flasks with and without macrophages. The bactericidal effect was determined by comparing the uptake with the actual number of live bacteria recovered within the macrophages at various times. Salmonella and E. coli strains opsonized with normal mouse serum differ both in their uptake by and survival within mononuclear cells. The most virulent Salmonella strain was phagocytosed and killed to a much lesser degree than the relatively avirulent E. coli strain. Using macrophages and serum obtained from mice which received Bacillus Calmette Guerin, 1 mg wet weight, intravenously 2-3 weeks previously, it was found that virulent Salmonella strains were phagocytosed and killed much more efficiently. These findings may provide a basis for the increased resistance of mice to Salmonella infection afforded by B.C.G. vaccination observed by Dubos and Schaedler.

20 *Immunology*

LYSIS OF THE STREPTOCOCCAL CELL WALL BY A PHAGE-ASSOCIATED LYSIN. Richard M. Krause and Maclyn McCarty. Rockefeller Institute, New York, New York.

Previous studies have shown that the cell wall of hemolytic streptococci is dissolved by a phage-associated lysin derived from the lysates of group C streptococci. Following lysis, the cell wall group-specific carbohydrate and the M protein are found in solution. In this study attention is directed to other products of cell wall lysis which are not antigenically defined. Trypsinized cell walls were lysed and the dialysable and non-dialysable soluble material as well as the insoluble residue were examined chemically. Almost all of the rhamnose was found in the non-dialysable fraction while less than one per cent was detected in the residue and the dialysable fraction. On the other hand, approximately 10 per cent of the total hexosamine was found in the dialysable fraction. The residue contained no hexosamine. The hexosamine in both the dialysable and non-dialysable fractions was found to be a mixture of glucosamine and muramic acid. Paper chromatography of the hydrolysed dialysable fraction reveals at least 4 amino acids, glutamine, alanine, lysine and glycine. These data suggest that as the result of cell wall lysis a dialysable mucopeptide is released which is chemically and serologically distinct from group-specific carbohydrate.

21 *Immunology*

CHEMICAL AND IMMUNOLOGICAL STUDIES OF PROTOPLAST MEMBRANES OF GROUP A STREPTOCOCCI. Earl H. Freimer* and Richard M. Krause. Rockefeller Institute, New York, New York.

The preparation of protoplasts of group A streptococci and the isolation of protoplast membranes has been described. These membranes are morphologically distinguishable from streptococcal cell walls, and are essentially free of cell wall carbohydrate and cytoplasmic nucleoprotein. Chemical analysis of the membranes indicates a composition of approximately 50 per cent protein and 25 per cent lipid. The high phosphorus content of this lipid suggests that phospholipid is its major component. Immunological studies on the protoplast membranes were performed with rabbit antisera prepared by immunization with intact membranes. Extracts of the membranes give strong precipitin reactions with membrane antisera, but only trace reactions with group A streptococcal antisera prepared against the whole streptococcus. Similarly, extracts of the streptococcal cell walls give a strong precipitin reaction with streptococcal antisera, but only trace reactions with membrane antisera. These results suggest that the membrane is chemically and antigenically distinct from the cell wall.

22 *Immunology*

THE USE OF CELLULOSE COLUMN CHROMATOGRAPHY FOR THE PURIFICATION OF THE HEAT LABILE TOXIN OF BORDETELLA PERTUSSIS. A. Banerjea* and J. Munoz. Stella Duncan Research Laboratories and Dept. of Microbiology & Public Health, Montana State University, Missoula, Montana.

Since the discovery of the heat labile toxin of B. pertussis by Bordet and Gengou, several attempts have been made to purify and characterize the toxin. Because of its great lability to heat on other agents, the purification has been difficult. The toxic fractions eluted from DEAE cellulose columns in the cold with a continuous gradient of NaCl in phosphate buffer contained primarily protein and little or no DNA, RNA or carbohydrate. A decreasing pH gradient in phosphate buffer was also found promising for the purification of the toxin. The absorption spectrum of toxic fractions gave peaks between 270 and 280 mµ and the toxicity of crude preparations as well as toxic fractions was completely destroyed by trypsin, but not by ribonuclease or desoxyribonuclease. It appears from the evidence obtained that the heat labile toxin is primarily a protein, although it is not possible to say at present whether other substances besides protein are present in the toxic molecule.
(Supported in part by NIH grant E-1832.)

158. MICROORGANISMS–Endotoxins

1 *Biochemistry*

FATTY ACID ESTER GROUPS IN LIPOPOLYSACCHARIDE ENDOTOXINS. Henry Tauber. Venereal Disease Exp. Lab., Communicable Disease Center, U. S. Public Health Service, School of Pub. Health, Univ. of North Carolina, Chapel Hill.

The lipid component is responsible for the toxicity of lipopolysaccharides. Thus the quantity of fatty acid esters present in endotoxins derived from different organisms became of interest. A recent assay for fatty acid esters in lipids (Snyder and Stephens, Biochem. et Biophys. Acta 34: 244, 1959) is applicable after minor modification. For analysis aqueous solutions of endotoxins were lyophilized and double quantities of reagents were used. Purple colored ferric hydroxamic acids derived from endotoxins have the same properties as those derived from lipids. They have an absorption maximum at 530 mμ. Optical density vs. concentration as found by the Beckman spectrophotometer over the concentration range 1 to 4 mg follows a straight line relationship. There are marked differences between the ester values of different endotoxins (N. gonorrhoeae 97, E. coli 08, S. abortus equi). There is no direct relationship between ester values and toxicities. The method offers an additional assay for the characterization of lipopolysaccharides.

2 *Biochemistry*

LIBERATION OF INTESTINAL SEROTONIN AFTER INJECTION OF BACTERIAL ENDOTOXIN. R.B. Davis, R.K. Ausman, W. R. Meeker, S.J. Gemmill, and J.B. Aust (intr. by C. J. Watson) Depts. of Medicine and Surgery, Univ. of Minnesota Medical School, Minneapolis.

Previous studies suggested that liberation of intestinal serotonin occurred after endotoxin injection (Davis, et.al. Circ. Res. In Press). To clarify these findings, pure E. Coli endotoxin (1 mg/kg) was injected into the superior mesenteric artery of the isolated, perfused small bowel of mongrel dogs. Samples were collected from the superior mesenteric vein. Initial platelet counts showed only small changes both after endotoxin and in control dogs. Free (plasma) serotonin was elevated in some specimens after endotoxin, but no elevation occurred after saline. Serum serotonin decreased slightly in control animals, but increased significantly after endotoxin. The average serotonin content of small bowel mucosa, relative to control levels, was higher after saline than after endotoxin, but the difference was not statistically significant. It is concluded that serotonin liberation from the small intestine occurs after endotoxin injection. (Supported by USPHS Grant H-5027, Am. Cancer Soc. and Minn. Heart Assn.

3 *Pharmacology*

ADRENERGIC INHIBITION AND THE LETHAL EFFECT OF BACTERIAL ENDOTOXIN IN MICE. R. A. McLean and L. Joe Berry *. Smith Kline & French Laboratories, Philadelphia, Pa., and Bryn Mawr College, Bryn Mawr, Pa.

The protective effects of various types of adrenergic inhibition have been tested against the lethal action of injected bacterial endotoxin in mice. Preliminary experiments substantiated the abilities of phenoxybenzamine hydrochloride to block the lethal effect of injected epinephrine and of cocaine to augment its toxicity. 3,4-Dichloroisoproterenol also increased the lethal effect of epinephrine. TM 10 or 2,6-xylyloxyethyltrimethylammonium bromide had no effect acutely but offered a low order of protection when given 24 hours before the epinephrine. Reserpine also protected when given 24 hours in advance. Iproniazid was without effect. The only compound to give significant protection against bacterial endotoxin was 3,4-dichloroisoproterenol. This substance has been shown by previous workers to block inhibitor and vasodilator effects of epinephrine. Such effects are probably metabolic in nature and may concern carbohydrate metabolism.

4 *Pharmacology*

STUDIES ON THE MECHANISM OF INHIBITION OF ARTHUS REACTION BY BACTERIAL CELL WALL COMPONENTS OF E. COLI. O. H. Ganley* and H. J. Robinson. Merck Institute for Therapeutic Research, Rahway, New Jersey.

Passive Arthus reaction was produced in rabbits and skin lesion area was measured 2 hours after injection of antigen. Agents under study were injected intravenously 1 hour before challenge. E. coli lipopolysaccharide and a subfraction of the lipopolysaccharide at low concentrations almost completely inhibited the Arthus reaction (control lesion, 12.8 cm^2; lipopolysaccharide, 20 γ/kg., 0.5 cm^2). Various other agents were also tested. Hydrocortisone and chlorpromazine at 10 mg./kg. caused partial suppression (41 and 50% respectively) while chlorpheniramine was inactive at this concentration. Since lipopolysaccharides are known to stimulate epinephrine secretion and to sensitize the vascular bed to the action of this hormone, it was postulated that the observed inhibition might be mediated through these hormones. Neither the ganglionic blocking agents hexamethonium and mecamylamine nor the adrenergic blocking agent Dibenzyline reversed the action of lipopolysaccharide or lipid. Further studies were carried out in adrenalectomized rats where lipopolysaccharide and lipid inhibited a formalin inflammation. The mechanism of action of lipopolysaccharides and lipids on the Arthus reaction will be discussed.

5 *Pharmacology*

INACTIVATION OF ENDOTOXIC POLYSACCHARIDE BY TISSUE HOMOGENATES. Vaman S. Waravdekar, Robert-John Trapani*, Maurice Landy*, and Murray J. Shear, Armed Forces Inst. of Path. Wash., D. C., and Nat. Cancer Inst., Bethesda, Md.

The blood of mammals has recently been shown to be capable of inactivating endotoxins in vitro. Animal tissues have now been examined for analogous potency to inactivate endotoxin. Rabbit liver was thoroughly perfused with chilled physiological saline to remove blood. Slices, homogenates or cell fractions were then incubated for one hour at 37°C with endotoxic polysaccharide (Serratia marcescens). The effect of these products on the endotoxin was shown by elimination of its capacity to elicit hemorrhagic necrosis in implants of Sarcoma 37 in mice. Liver slices did not affect the induction of tumor damage by endotoxin; however, homogenates prepared in a Waring blender did inactivate endotoxin. Comparative observations on the subcellular constituents of homogenate revealed that the combination of microsomes and supernatant inactivated endotoxin, whereas single constituents, including nuclei and mitochondria, did not possess this property. The conditions required for inactivation of endotoxin by tissue homogenate were different from those reported for the two systems in blood (J. Exp. Med. 108: 70, 1958; J. Clin. Invest. 39: February 1960).

6 *Pathology*

EFFECT OF THOROTRAST ON VASOMOTOR CHANGES DURING THE GENERALIZED SHWARTZMAN REACTION. Ting Chao Wong*, Michael Galton* and Donald McKay. Harvard Medical School, Boston, Mass.

To determine the nature of the state of "preparation" by Thorotrast for the generalized Shwartzman reaction the following experiments were performed. 1.5 to 2 kilo rabbits were pretreated intravenously with Thorotrast in a dose of 1.5 cc. per kilo. 3 hours later 0.2 mg. of endotoxin were given intravenously. They were then injected by aortic catheter with 10 cc. of a 10% suspension of India ink in saline at 2,4, and 6 hours after endotoxin injection. The animals were sacrificed immediately and autopsied. 30 micra unstained paraffin sections were examined microscopically. After Thorotrast preparation endotoxin produces glomerular capillary dilation, between 2 to 4 hours after endotoxin injection. Capillary dilation precedes fibrin deposition in glomeruli. Thorotrast plus endotoxin also produces central lobular "ischemia" of the liver. However, Thorotrast by itself does not cause these changes. Fibrin thrombi do not usually appear in organs that do not show a prior vasomotor dilation and they usually appear where vasomotor reaction takes place. Glomerular capillary dilation occurs under all conditions of "preparation" for the generalized Shwartzman reaction, hence plays an important part in "preparation" for the reaction. Supported by USPHS Grants H-4683 and C-2451.

7 *Pathology*

VASOMOTOR CHANGES IN THE PREGNANT RABBIT INDUCED BY BACTERIAL ENDOTOXIN. Michael Galton*, Ting Wong* and Donald McKay. Harvard Medical School, Boston, Mass.

Vasomotor changes in small vessels have been observed prior to the deposition of fibrin thrombi under certain conditions of preparation for the generalized Shwartzman reaction. It has been the purpose of this study to determine if such changes precede the thrombi when pregnancy is the preparing factor. Pregnant rabbits were given 0.2 mg. endotoxin intravenously and injected, 2,4 and 6 hours later, with 10 ml. India ink by an aortic catheter. The animals were sacrificed immediately and the tissues fixed in Formalin. 30 micra sections,unstained, were examined microscopically. 2 to 4 hours after endotoxin the glomerular capillaries were grossly dilated and packed with ink. The lungs contained large "ischemic" areas devoid of ink alternating with blackened areas containing markedly dilated ink-filled capillaries. The liver showed ink confined to dilated periportal sinusoids. The sinusoids of the adrenal and splenic pulp were dilated and filled with ink. Ink distribution in other organs did not differ from control animals. It is concluded that capillary dilatation precedes the deposition of fibrin thrombi in all the organs characteristically affected in the Shwartzman reaction in pregnant rabbits and thus plays an important part in "preparation" for the reaction. Supported by USPHS Grants H-4683 and C-2451.

8 *Pathology*

EFFECT OF PREGNANCY ON THE DISSEMINATED THROMBOSIS CAUSED BY BACTERIAL ENDOTOXIN. Donald McKay, Ting Wong*, and Michael Galton*. Harvard Medical School, Boston, Mass.

Differences have been observed in the specific localization of fibrin thrombi in rabbits given bacterial endotoxin intravenously when "preparation" for the generalized Shwartzman reaction was achieved by cortisone rather than a preceding injection of endotoxin. This study was done to determine if "preparation" by means of pregnancy alters the localization of the thrombi. In the non-pregnant animal after the second injection of endotoxin, thrombi were found in the renal glomeruli (53%), sinusoids of the red pulp of the spleen (64%), lung vessels (76%) and in the central veins of the liver (69%). They were not observed in the adrenal. After the first injection in pregnant animals thrombi were found in the renal glomeruli (82%), Malpighian corpuscles of the spleen (64%), lung vessels (88%), peripheral sinusoids of the liver (76%) and adrenal sinusoids (41%). "Preparation" of pregnancy shifts the localization of thrombi from the central vein to the periportal sinusoids of the liver, from the sinuses of the red pulp to the Malpighian corpuscles of the spleen and causes thrombi to appear in the adrenal. Specific localization of fibrin in the experiments on pregnant rabbits coincides precisely with that in the spontaneous generalized Shwartzman reaction in the pregnant human. Supported by USPHS Grants H-4683 and C-2451.

9 *Pathology*

EFFECT OF STEROID HORMONES ON THE RESPONSE TO BACTERIAL ENDOTOXIN. Alan B. Cooper* and Donald G. McKay, Harvard Medical School, Boston, Mass.

The ability of both cortisone and pregnancy to "prepare" rabbits for the generalized Shwartzman reaction suggested that the state of "preparation" might be mediated by other steroids and further that in pregnancy the sex steroids might play a major role. 25 mgm. of aqueous progesterone (proluton and lyolutin), estrogen (diethylstilbestrol in ethyl oleate), and aqueous testosterone were injected I.M. each day for 3 days into weanling rabbits. On the 4th day 0.2 mgm. of endotoxin were injected I.V. This procedure was repeated using estrogen (12.5 mgm.) plus progesterone (12.5 mgm.) in adult and weanling rabbits. Microscopic examination for fibria thrombi revealed that: 1) Progesterone, estrogen and testosterone, in the same doses as cortisone, do not prepare rabbits for the generalized Shwartzman reaction; 2) Progesterone alone greatly reduces the incidence of thrombosis; 3) Estrogen given concomitantly with progesterone counteracts the effect of progesterone and thrombi appear in the spleen in large numbers; and 4) Testosterone and estrogen increase the incidence of splenic thrombosis and decrease that of hepatic and pulmonary thrombi when compared to the control series with one injection of endotoxin. Supported by USPHS Grants H-4683 and C-2451.

10 *Pathology*

ADRENAL GLAND AND ADRENAL HORMONES IN REACTIONS TO ENDOTOXINS. R.A. Good and S.J. Zak*, Univ. of Minnesota Med. School, Pediatric Research Laboratories, Minneapolis, Minnesota.

Studies were performed to clarify role of adrenal gland and adrenal hormones in reactions to endotoxins in rabbits. Numerous corticoids had same effect as cortisone in promoting necrotizing effects of endotoxin. Treatment of rabbits with hydrocortisone provided at the time of or as late as 2 hours following administration of meningococcal or E.coli endotoxin protected against death. Single large lethal doses of endotoxin often produced bilateral adrenal hemorrhage and necrosis similar to that observed in human Waterhouse-Friderichsen syndrome. Treatment with adrenal hormone at time of or following endotoxin injection did not prevent these lesions. Complete adrenalectomy rendered rabbits susceptible to death with small doses of endotoxin. Small amounts of adrenal hormones restored resistance of adrenalectomized rabbits. Adrenalectomized rabbits manifest febrile response, develop local and generalized Shwartzman reactions, leukopenia, and refractoriness following injections of endotoxins. Cortisone treatment prevents development of refractoriness. Rabbits treated with cortisone for two weeks appear to show post-therapy hypoadrenal state correctable with small amounts of cortisone. (Aided by grants from U.S.P.H.S., Minnesota Heart and American Heart Associations)

11 *Pathology*

INFLUENCE OF ENDOTOXIN ON THE PLACENTAL-FETAL BARRIER. William M. McKell*, Hovald K. Helseth*, and Joel G. Brunson. Path. Dept., Univ. Miss. Med. Center, Jackson, Miss.

The effects of endotoxin on the placental-fetal barrier were studied in rabbits at varied periods of gestation. The animals were given an intra-arterial injection of 100 mcgm. of endotoxin by means of a polyethylene catheter inserted into the left femoral artery, extending to the bifurcation of the aorta. The catheter was left in place, and 5 ml. of a mixture of 2% trypan blue and colloidal iron were injected into it 4 hours after the administration of toxin. The animals were killed 30 minutes to 1 hour after the dye materials were given Control animals were given an intra-arterial injection of isotonic saline, followed in 4 hours by an injection of trypan blue-colloidal iron mixture. Microscopic examination showed the presence of the colloidal materials within the vascular channels of the placenta in both the experimental and control animals. In those given endotoxin, however, the dyes were present also in the vessel lumina of the fetal organs. The organs most consistently involved were the liver, kidneys, and cerebral and choroidal capillaries. In only one control animal was dye observed to have passed the placental-fetal barrier. The optimal period for passage of the colloids appeared to be from 21 to 29 days of gestation.

12 *Immunology*

TISSUE PERMEABILITY CHANGES PRODUCED BY B. PERTUSSIS IN MICE. J. Munoz. Stella Duncan Research Laboratories and Dept. of Microbiology, Montana State University, Missoula, Montana.

It is well known that Bordetella pertussis cells induce in mice changes in sensitivity to various drugs, infection and stresses including anaphylaxis (Kind, L. Bact. Rev. 22: 173, 1958). The mechanism by which B. pertussis induces these changes has not been uncovered and it is still a topic of speculation. In the present work it was found that B. pertussis cells produce in mice an increase in tissue permeability demonstrated one day after B. pertussis injection and lasting for at least eight days. Although reasonably good correlation was found between development of histamine sensitivity and increase of permeability in B. pertussis-treated mice, it was shown that other agents (S. enteriditis endotoxin or B. pertussis endotoxin) which do not sensitize mice to histamine also increase permeability. In addition, a strain of mice that does not sensitize to histamine after the injection of B. pertussis exhibits the same increase in tissue permeability. The changes observed may play a significant role in other effects produced by B. pertussis. It is postulated that the tissue permeability change observed may be responsible, at least in part, for the adjuvant effects of B. pertussis cells and endotoxins from other gram negative bacteria. (Supported by N.I.H. Grant E-1832.)

/3 *Immunology*

STUDIES ON THE RELATIONSHIP BETWEEN CHEMICAL COMPOSITION AND BIOLOGICAL PROPERTIES OF ENDOTOXINS. E. Ribi*, W. Haskins*, K. Milner* and M. Landy. Rocky Mountain Lab., NIAID, Hamilton, Mont. and Lab. Chem. Pharm., NCI, Bethesda, Md.

We have recently reported methods for the preparation of endotoxins with relatively low content of bound lipid, enabling us to investigate the concept of Westphal et al. that the host reactive properties of endotoxins are attributable primarily to their lipid moiety. Endotoxin preparations containing 19.2 and 1.2% firmly bound lipid respectively were obtained from the same harvest of Salmonella enteritidis by the Boivin procedure (extraction with trichloroacetic acid) and by our method (aqueous ether extraction). The capacity of these two preparations to evoke the various host responses characteristic of endotoxins e.g.: pyrogenicity, primary inflammation of rabbit skin, preparation for the dermal Shwartzman reaction, and lethality for thorotrast prepared rabbits; hemorrhagic necrosis in Sarcoma 37, stimulation of non-specific resistance to infection, and lethality for mice, was determined in parallel dose-response assays. Both products displayed high potency and did not differ significantly, nor did they differ in the way in which they were inactivated by interaction in vitro with EDC in human plasma. Consequently, the capability to evoke endotoxic reactions in the host was not dependent on the quantity of firmly bound lipid in the endotoxin complex.

/4 *Immunology*

PYROGENIC TOXINS OF THE GROUP A STREPTOCOCCI. Natalie Cremer* and Dennis W. Watson. Dept. of Bact. Univ. of Minn., Mpls.

The literature, past and present, is replete with reports on the endotoxin of gram negative bacteria. Recently the occurrence of a similar streptococcal endotoxin was reported (Stetson, J. Exp. Med., 104:921-934, 1956). The present report concerns the pyrogenic activity of sonic extracts of several types of group A streptococci in their relationship to one another and to group C streptococci, gram negative rods and extracellular products of T18. Dosage was determined by total N content of the crude extracts. Complete cross tolerance occurred among the group A streptococci, NY5, T12 and T18. The cross tolerance was not as complete between the group A streptococci and the gram negative rods. Differences in tolerance were noted, depending upon the streptococcal type and the gram negative bacterial species employed. The concentrated extracellular products of T18 contained a potent pyrogenic toxin. There was complete lack of cross tolerance between this toxin and the endotoxin of S. typhosa. A partial tolerance was observed between the intracellular products of T18 and its extracellular products. These data suggest two separate streptococcal pyrogenic toxins, one intracellular, the other extracellular. The extracellular toxins were described previously (Watson, J. Exp. Med., in press). A true evaluation of the relationship between these toxins awaits their isolation.

159. MICROORGANISMS—Luminescence

/ *Physiology*

PARTIAL PURIFICATION AND PROPERTIES OF THE LUMINESCENT SYSTEM OF THE FISH, APOGON ELLIOTI. Ed. H.-C. Sie*, F. H. Johnson and Y. Haneda*. Princeton University, Princeton, N. J., and Yokosuka City Museum, Japan.

The luciferin contained in excised, dried, and defatted internal photogenic organs of Apogon ellioti is readily extracted by cold methanol and remains stable in a dried residue. Like luciferin of the Cypridina system, with which components of the Apogon system cross-react, Apogon luciferin in aqueous solution undergoes an initially reversible dark oxidation by oxygen or ferrycyanide, but decomposes on standing. Apogon luciferin can be partially purified by Anderson's benzoylation procedure, though with poor yield. High yield is obtained by anaerobic chromatography on a cellulose column with Tsuji's solvent, as used with Cypridina. The active fraction is yellow fluorescent and yellow colored, with absorption peaks at 425 (weak) and 258 (strong) millimicrons. During dialysis, Apogon luciferase looses activity in a manner initially reversible by adding dialysate, whereas Cypridina luciferase withstands exhaustive dialysis. Supported by ONR and NSF.

2 *Biochemistry*

ON THE NATURE OF THE NUCLEOTIDE REQUIREMENT IN LUMINOUS EXTRACTS OF RENILLA RENIFORMIS. Milton J. Cormier(intr. by William L. Williams). Dept. of Chemistry, Univ. of Georgia, Athens.

It has previously been reported that extracts of the sea pansy, R. reniformis, require Renilla luciferin and luciferase, oxygen, and either ATP, ADP, or AMP for luminescence. Since firefly luminescence requires a primary activation by ATP, the possibility that both systems were ATP activated appeared to be a good one. This has been found not to be the case on the basis of inhibitor studies and recent experiments on the purification of Renilla luciferase. For example, the ATP-stimulated reaction is partially inhibited by fluoride and is metal activated, whereas fluoride or metals have no effect on the ADP or AMP-driven reactions. ATP functions indirectly in this system as shown by kinetic measurements. Furthermore, ATP acts as a generating system for ADP and/or AMP since purified enzyme fractions that are devoid of ATPase activity do not respond to ATP, although such fractions still luminesce in the presence of luciferin and ADP or AMP. It is concluded that the nucleotide required for sea pansy luminescence is either ADP or AMP, but not ATP. The mechanism of nucleotide action is being investigated. (Supported by a grant from the National Science Foundation.)

3 *Biochemistry*

PURIFICATION AND PROPERTIES OF GONYAULAX LUCIFERIN. Vernon C. Bode * and J. Woodland Hastings. Division of Biochemistry, Univ. of Illinois, Urbana

Bioluminescence in cell-free extracts of the marine dinoflagellate, Gonyaulax polyedra, requires an enzyme (luciferase), a heat stable dialyzable substrate (luciferin), oxygen, and relatively high salt concentrations (1M). Luciferase has been purified by ammonium sulfate fractionation and is stable for months at -10°C. Luciferin obtained from a hot water extract of the cells rapidly loses activity, even at -10°C. This nonenzymatic destruction is due at least partly to a nonluminescent oxidation, since the activity of dark luciferin preparations may be partially restored by certain reducing agents. Luciferin is tightly bound to crystalline bovine serum albumin (BSA), and luciferin bound to BSA is more stable to dark destruction. Proteins such as γ-globulin and ovalbumin will not replace BSA in this respect. Salt also promotes the nonluminescent destruction of luciferin. In 1M salt luciferin loses 50% of its activity within 10 minutes. During luminescence, therefore, luciferin is being destroyed via both luminescent and nonluminescent pathways, and changes in the yield of the reaction in the presence of various added compounds can be attributed to differential effects upon these pathways. Thus, cysteine, BSA, and histidine added to the reaction result in a marked increase in luminescence.

160. MINERAL METABOLISM

/ *Physiology*

OSMOREGULATION IN THE LAND CRAB, CARDISOMA GUANHUMI. I. REGULATION IN DILUTE SEA WATER. Charles E. Lane, Charles A. Gifford* and Francis J. Bernard*, The Marine Laboratory, University of Miami, Miami, Florida.

The land crab, Cardisoma guanhumi survives for months when allowed access only to 0.5% sea water but tolerates 175% sea water for less than seven days. Serial blood, urine and stomach fluid analyses for Na, K, Ca, Mg, SO_4, NH_4, Cl and total osmotic pressure, were made on a group of twelve animals in 0.5% sea water at irregular intervals over a total period of 100 days. Na, K, Mg, Cl and total osmotic pressure are well regulated throughout the hypotonic range. Ca and SO_4 appear to illustrate a different regulatory mechanism. Stomach fluid is hypertonic to blood and urine for all ions examined except Na. The sensitivity of the ion regulating mechanisms is illustrated by the relative constancy of hemolymph concentration over the salinity range fresh water to sea water. (Supported by a grant from The National Science Foundation).

2 *Physiology*

LOCATION OF IONIC TRANSFER IN THE DOG PAROTID GLAND. L. L. Langley and R. S. Brown.* Dept. of Physiology, University of Alabama School of Dentistry, Birmingham, Alabama.

The stop-flow procedure has been used to determine the specific location of ionic transfer in the dog parotid gland. In all cases a flow of approximately 20 drops per minute was evoked by i.v. infusion of pilocarpine. In one series after the flow had been stopped by closing a valve on the outlet tube, K^{42}, Na^{24}, Ca^{45}, I^{131}, and Cl^{36} were injected into the ipsilateral carotid artery. Forty-five seconds later the saliva was collected in two-drop samples. The plotted results show maximal transfer into the saliva at specific locations in the gland. Calcium peaks lowest in what is assumed to be the most distal portion of the collection ducts. Sodium and chloride show peaks about 4 drops higher; and potassium about 2 drops still higher. The maximal transfer of iodine occurs still higher in a region that may approach, or include the acinar cells. In the second series, after a constant blood concentration of the isotope had been achieved, a stop-flow analysis was carried out with an occlusion of 90 seconds. There is an increased concentration of calcium and potassium low in the collecting ducts and a decreased concentration of sodium and chloride at the same location. Iodide increases higher over a broader range suggesting acinar concentration. (Supported by NIH Grant No. D-329(C3).

3 *Physiology*

K AND Na OF RAT DIAPHRAGM AS AFFECTED BY $HgCl_2$ IN VITRO. Eleonore A. Ohr (intr. by A.B. Craig, Jr.) Univ. Rochester Sch. Med. & Dentistry, Rochester, New York.

Hemidiaphragms from 100 gm rats were incubated in Krebs-Ringer-phosphate solution at 37° C for given intervals up to 3 hrs. Movements of Na and K as induced by varying concentration of $HgCl_2$ were followed. The control hemidiaphragm was from the same rat as the experimental and was treated in the same manner except that $HgCl_2$ was omitted from the medium. Changes in concentration of Na and K occurred only within the first 30-40 min.; the equilibrium value of Na was attained prior to that of K. Plotting the equilibrium value of $\Delta Na/\Delta K$ (Δ = expt.-control) results in a curve levelling off at 1.1. $\Delta K = -1.3\Delta Na-9$. This is thought to indicate that there are at least two mechanisms involved in the action of $HgCl_2$ on muscle Na and K. In contrast with the changes of K and Na, the release of UV-absorbing material from the cell is continuous throughout the experimental period. This release is accelerated by the presence of $HgCl_2$ in the medium.

4 *Physiology*

MANGANESE-PHENOTHIAZINE COMPLEXES AS MODELS OF SPECIFICITY IN METAL BINDING. D. C. Borg* and G. C. Cotzias. (intr. by S. Cohn.) Med. Res. Center, Brookhaven Natl. Lab., Upton, N.Y.

We have shown earlier (Fed. Proc. 17:430,1958) that chlorpromazine (CPZ) forms coordinate complexes with manganese ions and that this might explain the diversity of the drug's actions. Since phenothiazines as a group exhibit most of the metabolic effects of CPZ, the metal binding observations already made with CPZ were extended and found to apply to a large number of phenothiazine compounds. Although Mn^{++} ions formed colored coordination compounds readily with all the phenothiazines tested (following aerobic titration with alkali and then back titration with acid), phenothiazine sulfoxides were almost inactive, just as they are almost inactive in vivo. Under the same conditions phenothiazines formed no demonstrable coordination complexes with other bivalent metals tested (including Fe^{++}), but Fe^{+++} complexes formed readily, even anaerobically and without titrations. The manganese and iron coordinates were spectrophotometrically identical. However, the difference in the redox potentials of the Mn^{++}/Mn^{+++} and Fe^{++}/Fe^{+++} couples (-1.51 v. and -0.77 v., respectively) emphasize the specificity of the phenothiazine reaction with Mn^{++}. The data may be explained by electron transfer processes that are generally applicable to other complexes exhibiting restricted metal requirements.

5 *Physiology*

THE GASTROLITHS OF THE CRAYFISH AS CONVENIENT SITES FOR THE STUDY OF MECHANSISMS OF MATRIX AND MINERAL DEPOSITION IN CALCIFIED STRUCTURES. Dorothy F. Travis (intr. by J. H. Welsh.) Biological Laboratories, Harvard University, Cambridge, Mass.

As gastrolith deposition begins, histological, histochemical, and ultrastructure results have shown that:1)Active epidermal cells concentrate large amounts of calcium;2)Alkaline phosphatase, protein and lipid become concentrated in the apical epidermal fibrillar processes;3)Glycogen reappears cyclically in the epidermis during matrix synthesis and calcification;4)Two types of submicroscopic material are secreted from the epidermis into the amorphoid matrix--granular particles which form small crystallites of calcite, these in turn forming small crystals; short beaded fibrils which first form loose organic crystalline-like structures. As these short fibrils polymerize to form fibrous lamellae of the matrix, granular centers are freed forming small crystals;5)The gastrolith matrix contains as its major ground substance a glycoprotein, an acid mucopolysaccharide, in close association with a lipid complex of glycerides, phospholipids, and sterols. Work in progress indicates that the gastroliths by weight consist of: 47.54% mineral; 52.46% organic; 1% or less protein; 8-10% lipid; and 3-4% sterols, most of which are esters. (Supported by a Special Fellowship from the National Heart Institute, U.S.P.H.)

6 *Physiology*

INTERMEDIARY CELLULAR METABOLISM RELATED TO ELECTROLYTES IN THE EXPERIMENTAL NEPHROTIC SYNDROME*. T. Yoshida, F. Yamashita, E. Kaiser, M. Piccardo and J. Metcoff (intr. by R. Levine) Michael Reese Hospital; Northwestern Univ. Med. Sch., Chicago.

Increased cell H_2O (ICW) & $[Na]_i$; decreased $[K]_i$, $[Mg]_i$ and $(P_o)_i$ characterize muscle of rats with the experimental nephrotic syndrome induced by injection of 6-dimethyl amino-purine riboside (aminonucleoside). A possible effect of these ionic alterations upon cellular metabolic sequences has not been explored. Pyruvate uptake from Krebs-Ringer media by isolated diaphragm from 12 nephrotic rats was 3x that of pair-fed controls (21.3 vs 7.1 μM/min/g non-collagen N). The effective ICW activities (mM DPN-H/min/L ICW) of lactic dehydrogenase (52 vs 62) and pyruvic kinase (4.6 vs 6.2), & concentrations of intermediates lactate (14.0 vs 17.6 mM/L), citrate (321 vs 420 μM/L) and α ketoglutarate (348 vs 462 μM/L) were decreased in skeletal muscle of nephrotic rats. Examples of relation between enzymic ion cofactor and intermediate (M/M) emphasize the significance of cellular electrolyte changes in muscle of Nephrotic vs Control: $(K)_i$/pyr 177 vs 279, $(Na)_i$/pyr 42 vs 9, $(Mg)_i$/pyr 25 vs 34, (K_i)/citr 358 vs 44, $(Na)_i$/citr 86 vs 15.

A new, dynamic reference base relating intracellular electrolytes to appropriate intermediary metabolites is suggested and indicates altered terminal metabolic pathways in muscle of nephrotic rats. (*Supported by USPHS A-1325 C2.)

7 *Biochemistry*

THE REGULATION OF IRON TRANSPORT IN THE GUT. Philip Charley* and Paul Saltman. Dept. Biochem., School of Med., Univ. So. Calif., Los Angeles.

The most widely accepted mechanism for the control of the iron transport from the lumen of the gut into the bloodstream is that of the "mucosal block." To critically examine this phenomenon, experiments were designed whereby Fe^{59}, either as ferrous or ferric ion, was placed in the gut; the blood was continually monitored and radioactivity determined in the circulating system. These studies revealed that no such phenomenon as a "mucosal block" exists either in anemic or normal rabbits. The iron placed in the lumen of the gut moves directly into the bloodstream with a lag equivalent to the amount of time necessary for the blood to reach the scintillation well counter. Both ferrous and ferric forms are readily transported. The only difference between ferrous and ferric ions seems to be in their solubilities. If specific chelates are added to stabilize the ferric form, it moves rapidly into the circulating system. The rate of transport and the total amount of iron ultimately reaching the blood are controlled by the degree of unsaturation of the circulating transferrin. Thus, in rabbits which are anemic, the rate of transport is fast and the total amount relatively large. The transport of iron ceases at that time when the iron binding capacity of the transferrin is essentially saturated.

8 *Biochemistry*

EVIDENCE OF AN IRON REQUIREMENT BY Entamoeba histolytica CULTIVATED IN A THREE-AMINO ACID MEDIUM WITH PENICILLIN-INHIBITED CELLS OF Bacteroides symbiosus. Nelda G. Latour (intr. by R. E. Reeves.) L. S. U. Sch. Med., New Orleans, La.

It has been possible to demonstrate an Fe requirement for the multiplication of Entamoeba histolytica in a medium containing cysteine, arginine, and glutamic acid. Good, continuous amoeba growth was obtained in this medium with $4x10^{-5}$ M Fe, but when the Fe was lowered to about $4x10^{-7}$ M, amoeba growth markedly decreased and was not continuous. The Fe in this medium could not be replaced by the cations of Co, Cu, Mn, Ni, or Zn. Amoeba multiplication was inhibited by adequate amounts of the ferrous iron chelators, chiniofon and o-phenanthroline, and a reversal of this effect could be accomplished by in turn increasing the Fe concentration. It is interesting to note that the ferric iron chelators, CHEL DP and Versene Fe-3 Specific, were not effective in inhibiting amoeba multiplication. It would appear that ferrous iron is necessary for amoeba growth in the three-amino acid medium.

Cation added to medium	Amoeba harvest, transfer 1	2
$4x10^{-5}$ M Fe	282,000	261,000
$4x10^{-7}$ M Fe	2,600	1,400
$4x10^{-5}$ M Co	33,000	0
$4x10^{-6}$ M Cu	0	0
$4x10^{-5}$ M Cu	1,700	0
$4x10^{-5}$ M Ni	2,800	0
$4x10^{-6}$ M Mn	1,800	0
$4x10^{-5}$ M Mn	0	0
$4x10^{-5}$ M Zn	0	0

9 *Biochemistry*

CATIONS OF THE CALF LENS. J. H. Kinoshita, H. L. Kern* and L. O. Merola*. Harvard Medical School and Mass. Eye and Ear Infirmary, Boston, Mass.

The factors which influence the cation content of calf lens were studied. The calcium level of the medium bathing the lens appears to be extremely important. A critical concentration of this cation in the incubating medium must be exceeded in order to maintain normal levels of Na and K in the lens. Exposure of lens to 0°C results in a loss of K and a gain of Na. Incubation of cold-exposed lens at 37°C restores the Na and K to normal levels. When the incubating medium contains glucose, the recovery process is not dependent on O_2; that is the Embden Meyerhof pathway appears sufficient to support the mechanism that extrudes Na and concentrates K. However, when the medium contains no glucose the lens requires O_2 for the partial recovery of cations observed. Apparently energy derived from aerobic but not from anaerobic utilization of endogenous substrates is sufficient to restore partially the cations to normal levels. The transparency and general appearance of the lens in these recovery experiments seem to reflect how successfully the electrolyte gradients have been reestablished. At 0°C the normally transparent lens is opaque, but after the temperature is raised to 37°C the opaque lens slowly regains its clarity. It seems that the state of transparency of the lens closely parallels the recovery of the cations.

10 *Pharmacology*

A SIMPLE METHOD OF MAKING GRADED COLLODION MEMBRANES. S. A. Peoples. School of Vet. Med., Univ. of Calif., Davis, Calif.

This method makes it possible to make self supporting collodion membranes of the desired pore size of any shape or size with sufficient strength to permit rapid ultrafiltration. Dissolve 10 gms of Parlodion in a solution of 50 ml ether and 50 ml of a mixture of absolute alcohol and propylene glycol. The amount of propylene glycol determines the pore size of the membrane. The solution is poured into the glass mold, such as a test tube, and when the bubbles have risen, the tube is inverted in an alcohol-ether atmosphere for 5 minutes. The tube is then inverted over propylene glycol in a closed container until the membrane is free of ether and alcohol and may be left until used. The membrane is best removed by first filling the tube with water for 30 minutes. Reuse is possible if stored in 60% ethyl alcohol. The radius of the pores bears a linear relationship to the propylene glycol/Parlodion ratio from 4 Å at 0.5 to 50 Å at 4.0. Comparison between batches of solution indicate a predictability of pore size of 10%. Flat sheets can be made by adding a measured amount of solution to a petri dish, spreading under alcohol ether and drying over propylene glycol.

11 *Pharmacology*

EFFECTS OF CHLOROTHIAZIDE ON BODY ELECTROLYTES. Donald A. Sones,* Khalil G. Wakim, Alan L. Orvis* and Warren F. McGuckin.* Mayo Clinic and Mayo Foundation, Rochester, Minn.

Oral administration of chlorothiazide to laboratory animals in moderate and large doses for 5 months produced marked increase of urinary sodium, chloride and, to a lesser extent, potassium. Urinary volume was consistently increased. Despite marked natruresis and chloruresis, blood levels remained within normal range. Furthermore, isotope studies utilizing K^{42} and Na^{24} did not reveal any significant difference in total body exchangeable potassium or sodium in the animals receiving chlorothiazide over long periods when compared with controls. Yet, hypokalemia was found consistently when large doses were given over long periods. No evidence of the hypokalemic state was detected electrocardiographically. Values for serum calcium and for magnesium in the plasma and erythrocytes were within normal range in both control and hypokalemic animals. Maintenance of normal magnesium levels may contribute to the relatively asymptomatic nature of chlorothiazide-induced hypokalemia and the absence of electrocardiographic abnormalities.

12 *Pathology*

KINETIC CRITERIA OF HOMEOSTASIS AS APPLIED TO ZINC AND CADMIUM METABOLISM. G.C. Cotzias and B.H.Selleck*. (intr. by L. K. Dahl.) Medical Res. Center, Brookhaven Natl. Lab, Upton, N.Y.

We reported last year that the tissues of the intact mouse (which distinguish precisely between manganese and other elements) confuse cadmium for zinc as indicated by small displacements of Zn^{65} by stable cadmium salts. It was thought that homeostatic responses to zinc or to cadmium, if existing, might be common to both elements. Two criteria of homeostatic response were sought: (1) The acceleration of total body turnover of isotopically tagged mice following administration of the stable elements; (2) The absorption of Zn and Cd radioisotopes from the gut as a function of parenterally given loads with the stable elements. Metabolic burdens with stable zinc caused marked acceleration of Zn^{65} turnover and marked diminution of Zn^{65} absorption, but had no effect on Cd^{109} turnover or absorption. Furthermore, the slow turnover and the absorption of Cd^{109} were insensitive to metabolic burdens with both cadmium and zinc salts. Thus there was revealed a fundamental difference between the metabolic pathways of cadmium and of zinc: no homeostatic control of cadmium could be discerned. At least two types of control of zinc were discovered: a negative feedback for its absorption and a positive feedback for its excretion. This indicated that some zinc-containing pools transmit homeostatic signals while the cadmium-containing ones do not.

13 *Pathology*

MANGANESE METABOLISM AND ADRENOCORTICAL ACTIVITY: AN ISOTOPIC STUDY. R. R. Hughes* and G. C. Cotzias. Medical Research Center, Brookhaven National Laboratory, Upton, L.I., N.Y.

Previous evidence has stressed the constancy of the concentration of manganese in tissues and the dependence of the turnover rates on the animal's intake of manganese. (Physiol. Reviews 38:503,1958.) Changes of the biological turnover of trace metals in whole animals and their organs might be used to explain hormonal action provided that (1) a significant response is discerned; (2) the dietary intake is constant. Thus several parameters of adrenal function were varied in mice on constant manganese intake and the effect on the metabolism of Mn^{54} was noted. Presently it appears that glucocorticoids accelerate the total body turnover rate of Mn^{54} while mineral corticoids either have no effect or possibly retard it. However, the partition of this element among the organs was affected by both sets of compounds in the same way. The most striking finding was the isotopic enrichment of the carcass and the depletion of the liver by both the mineralocorticoids and glucocorticoids. These findings lend validity to correlation of metabolic properties of manganese and of adrenocortical steroids. The correlations can be reconciled by an assumption which is supported by the chemical structure of these steroids, namely that they are metal ligands. Metal binding can explain steroid action at least in part.

14 *Pathology*

NEUTRON ACTIVATION DETERMINATION OF MANGANESE IN BODY FLUIDS. P. S. Papavasiliou* and G. C. Cotzias. Medical Research Center, Brookhaven National Laboratory, Upton, L. I., N. Y.

Effective amplification of signals originating in an element is critically needed in trace metal studies. Bowen (J. Nucl. Energy 3:18,1956) has shown that the intense radioactivity of the isotope Mn^{56} (which may be produced in a reactor from the natural Mn^{55}) satisfies this need in the case of a reticent element. Blood is a good test tissue in this context because of its clinical importance and its paucity in Mn. 0.05 ml samples were placed on prepared filter paper, dried and neutron-activated in a reactor of a total neutron flux 1.2×10^{13} for 2.5 hours. It was then flash-burned in O_2 and the ashes were collected in acid containing 2.0 mg of Mn carrier. The element was oxidized to permanganate with ammonium persulfate in the presence of Hg and Ag as catalysts. The permanganate was precipitated with tetraphenylarsonium chloride. The precipitate was washed to free it from other coactivated elements. Its γ-radioactivity and dry weight were determined. Thus the specific activity of the carrier was calculated. Identical handling of known traces of Mn provided the standards from the specific activity of which the Mn concentration in the blood was calculated. This simple procedure was also applied to other fluids of biological interest following various biological maneuvers affecting the behavior of manganese.

15 *Nutrition*

EFFECT OF COOKING ON CA^{45} AND SR^{89} IN BEEF LOIN ROASTS. M. C. Bell and R. G. Buescher*. UT-AEC Agric. Res. Lab., Oak Ridge, Tenn.

Boneless roasts and roasts with bone were obtained from each of 16 steers dosed orally with 12 mc Ca^{45} and 6 mc Sr^{89}. The roasts were cooked in heavy aluminum foil in an oven preheated to 325° F. When the temperature reached 160° F in the center of the roast as shown by a meat thermometer, the roasts were removed from the oven. The boneless roasts showed 25% cooking loss compared to 19% for those with bone. Meat samples from the roasts with bone were significantly higher in Ca^{45} and Sr^{89} content than from boneless roasts. Ca^{45} and Sr^{89} content of the meat near the bone was also significantly greater than meat fartherest from the bone in the cooked roasts. Drippings from the roasts with bone contained significantly more Ca^{45} and Sr^{89} than drippings from the boneless roasts. Uncooked bone contained 3.1×10^3 as much Ca^{45} and 1.7×10^3 as much Sr^{89} as uncooked meat from these roasts.

These data show that Ca^{45} and Sr^{89} moved from the bone into the meat and drippings upon cooking of the beef loin roasts.

16 *Nutrition*

INFLUENCE OF STABLE DIETARY BARIUM, STRONTIUM AND CALCIUM ON UPTAKE OF BA^{133}, SR^{89}, CA^{45}, STABLE STRONTIUM AND CALCIUM BY TIBIAE OF CHICKS. Frank R. Mraz. UT-AEC Agric. Res. Lab., Oak Ridge, Tenn.

Two hundred and twenty-five New Hampshire cockerel chicks were divided into 15 groups of 15 each and fed diets containing varying levels of stable strontium (0.0 - 1.09%), barium (0.0 - 0.86%) and calcium (1-2%) ad libitum for a period of 3 weeks before dosing. A three dimensional central composite design described by Box (Biometries 10, p.16, 1954) was used. An oral dose of Ca^{45}, Sr^{89} and Ba^{133} was administered to 10 birds from each group and the remaining 5 birds were saved for stable calcium and strontium determination. All birds were sacrificed 48 hours after dosing and tibiae removed for radioactive assay. As the level of dietary calcium increased, Ca^{45}, Sr^{89} and Ba^{133} deposition per gram of ashed tibia decreased. Conversely, as the level of dietary barium rose, the content of these radionuclides in the tibiae also rose. Dietary strontium increased Ba^{133} deposition but had no consistant effect on Ca^{45} or Sr^{89} deposition. As dietary strontium increased, the stable strontium per gram of ashed tibia increased and the stable calcium decreased.

161. MINERAL METABOLISM– Calcium, Phosphorus

1 *Physiology*

ION ASSOCIATION. I. THE EFFECT OF UNIVALENT AND MULTIVALENT ANIONS ON THE CONCENTRATION OF FREE CALCIUM IONS IN SERUM ULTRAFILTRATE. Mackenzie Walser. Johns Hopkins School of Medicine, Baltimore, Maryland.

The existence of electrostatic ion-pairs in body fluids follows from physicochemical considerations. We have reported elsewhere that alkaline earth cations (Ca, Mg, Sr) when thus paired with multivalent anions (SO_4, S_2O_3, HPO_4) have altered biological properties. A complete analysis of the ionic composition of body fluids therefore requires estimation of free and paired ion concentrations. Free calcium and magnesium ions may now be determined spectrophotometrically. Paired (or complexed) ions may be estimated from anion concentrations using known dissociation constants. This scheme was tested by employing normal serum ultrafiltrates, in which ion association is minimal compared to that in urine or cell fluid. Multivalent anions (SO_4, HPO_4, $Fe(CN)_6$) were added to serum or serum ultrafiltrate as sodium salts at constant ionic strength (.16). Free calcium ion concentration in ultrafiltrate, measured by the purpurate method, (corrected for dilution) decreased 10 to 50%. Dissociation constants derived from these data (at 25°C) were as follows: $Ca:HPO_4$, 0.026M; $Ca:SO_4$, 0.05M; $Ca:Fe(CN)_6^{2-}$, 0.015M. These values correspond closely to reported physicochemical data.

2 *Physiology*

SIGNIFICANCE OF COMPARATIVE Sr^{85} AND Ca^{45} PLASMA VALUES IN MAN. Joseph Samachson and Berta Spencer (intr. by Henry Haimovici.) Montefiore Hospital, New York.

Statistical analyses of Sr^{85} and Ca^{45} plasma levels of patients following intravenous and oral administration of both tracers are of physiological interest. They were simultaneously administered orally on 24, and intravenously on 9 occasions. Also, 48 intravenous and 18 oral doses of Sr^{85} alone and 2 oral doses of Ca^{45} alone were administered. After intravenous administration of Sr^{85} and Ca^{45}, plasma curves of the first day are not quite identical. Ca^{45} values average 5% lower at 1 hour, indicating more rapid passage of Ca^{45} into interstitial fluid. However, Ca^{45} values decrease more slowly and are 6% higher at 24 hours. After 2 weeks, because of greater Sr^{85} excretion, Ca^{45} values are much higher than those of Sr^{85}. Ca^{45} is absorbed more rapidly and more completely than Sr^{85}. Usually, complete absorption requires 4 hours, occasionally 8 hours. Neither isotope was absorbed between the 8th and 24th hour. Different areas of the small intestine seem to absorb the 2 radioisotopes in different ratios. Calculations made from plasma levels after oral and intravenous administration to the same patient give values of intestinal absorption of Sr^{85} and Ca^{45} in good agreement with data obtained from absorption studies. (Supported by a grant from the U.S. Atomic Energy Commission.)

3 *Physiology*

DIFFERENCES BETWEEN ACCRETION AND RESORPTION IN BONE CELL METABOLISM. George Nichols, Jr., Nancy Nichols* and André B. Borle*. Harvard Med. School, Boston, Mass.

Continuous remodeling in adult bone without change in size implies an overall metabolic pattern which is the net result of equal and opposite processes of accretion and resorption. To examine the differences between these two, we have studied O_2 and glucose consumption, and lactate and citrate production in vitro by metaphyses from normal, parathyroid extract, and estrogen treated adult male mice. Aerobically in HCO_3^- buffer lactate production was large - 73 times citrate - and increased 35% compared to controls following PTE (3.44 vs 2.56 μM/mg cell N/hr.) while citrate, glucose and O_2 did not change significantly. Estradiol decreased lactate slightly without effect on glucose or O_2. Anaerobiasis, Pi buffer or lack of substrate abolished PTE effect. A Pasteur phenomenon appeared only in estrogen group. Correlation of glucose utilization with lactate production indicated lactate produced was greater than glucose consumed in PTE group; accounted for 84% of glucose in normals; and was less than glucose used after estrogen. Thus brisk aerobic glycolysis is common to both processes; differences between metabolism in accretion and resorption are largely quantitative rather than qualitative. (Supported by U.S.P.H.S. Grants A-854 (C3 & 4).

4 *Physiology*

DIFFERENTIAL EFFECTS OF IMMOBILIZATION IN CALCIUM AND STRONTIUM METABOLISM. Felix Bronner and Paul D. Saville* Hospital for Special Surgery, Cornell Medical Center, New York 21, New York.

Ca, P, N balances and the fate of I.V. injected Ca^{45} and Sr^{85} were studied in two patients with scoliosis before and after immobilization. Immobilization caused the urinary Ca output to rise from a mean of 340 mg/24 hrs. (36 days) to a mean of 376 mg Ca/24 hrs (92 days) in the 17-yr-old boy, and from a mean of 179 mg/24 hrs (30 days) to an average of 226 mg/day (27 days) in the 16-yr-old girl. Immobilization produced no significant changes in the urinary excretion of N and P, but induced a drop in the creatinine output. The 6-day cumulative Ca^{45} output in urine rose from an average (2 trials) of 18% of dose to 38% (1 trial) in the boy and from an average of 20% of dose (2 trials) to 27% (1 trial) after immobilization. Notwithstanding these changes in urinary specific activity, which suggest alterations in the rates of bone formation and resorption, there were no corresponding changes in Sr^{85} excretion; it equalled approximately 50% both before and after immobilization in the boy. In the girl Sr^{85} excretion rose from a mean of 46 to 57%, a rise proportionately less than that for Ca^{45}. These results suggest qualitative differences in the manner in which these minerals enter, and/or are released from, bone.

5 *Physiology*

THE EFFECT OF HIGH CALCIUM INTAKE ON CALCIUM METABOLISM OF MAN. Margaret Li, Herta Spencer and Joseph Samachson (intr. by Morton Galdston.) Montefiore Hospital, New York.

The effect of calcium added to a standard low calcium diet (150 mg Ca) was studied under controlled dietary conditions on intestinal absorption and on the excretion of calcium in 30 patients ranging in age from 20 to 75 years. Metabolic balances were performed during low and high calcium intake, the latter representing a 6-10-fold increase over the former. Added calcium was given for 10-690 days prior to studying the effect of high calcium intake. The utilization of additional calcium was considerably lower in older persons and in those with osteoporosis of the senile or post-menopausal type than in young persons. Studies using tracer doses of Ca^{45} and/or Sr^{85} orally during low and high calcium intake revealed that the absorption of the radioisotopes remained either unchanged or decreased during high calcium intake and that this change may depend on the duration of the high calcium intake. The increase of urinary Ca^{45} and Sr^{85} excretion corresponded to that of urinary calcium which, however, was frequently only small and correlated with poor intestinal absorption of added calcium. (Supported by grants from the U.S. Public Health Service and the U.S. Atomic Energy Commission.)

6 *Physiology*

EFFECT OF pH ON THE ULTRAFILTRABLE CALCIUM FROM RABBIT SERUM. Neal A. Peterson* and J. M. Crismon. Dept Physiology, Stanford University School of Medicine, Stanford, California.

Manipulation of pH in vivo and in vitro was followed by determination of pH, total calcium, (TCa) and total protein concentrations (TPr) on the separated serum samples from rabbit blood. In addition, ultrafiltrates, obtained anaerobically, were analyzed for calcium. The McLean & Hastings[1] equilibrium constant (K) was found to be linearly related to pH over a wide range of pH values in the following form: $pK=0.588pH-2.544$. Additional relationships were derived for the combined effect of pH, TCa, and TPr on the change in (Ca^{++}) per unit change in pH (dCa^{++}/dpH). A set of nomograms prepared from calculations using all three variables gave plots of (Ca^{++}) against pH at various TCa isopleths, each nomogram of the set being specific for a given (TPr). The information from the nomograms was resolved into a simple linear equation giving (Ca^{++}) in terms of serum TCa, TPr, and pH: $(Ca^{++})=TCa-TPr(0.0182pH\ TCa + 0.0784pH-0.108TCa-0.536)$. Separate analyses of sera for pH, total Ca, total protein and ultrafiltrate Ca were employed to test the equation. The average deviation of values of (Ca^{++}) calculated from pH, TPr, and TCa, from those obtained by analysis of serum ultrafiltrates was 0.09mM/L.

1. McLean, F.C. & Hastings, A.B., J. Biol. Chem. 108:285, 1935.

7 *Physiology*

METABOLIC STUDIES ON ALUMINUM GEL PHOSPHORUS DEPLETION. S. H. Lau*, A. G. Goldman* and J. Grossman. Montefiore Hospital, N.Y.

In attempts to study effects of P deprivation on parathyroid function, aluminum hydroxide was given to 11 patients under metabolic control. Six were normal subjects, three were suspected of having hyperparathyroidism because of renal calculi and low or borderline serum P levels, but not verified, one had idiopathic hypoparathyroidism, and one, generalized hypoendocrinism including hypoparathyroidism. Most normal subjects manifested the expected increased fecal and decreased urinary P excretion with negative P balances, progressive fall in serum P, and increased fecal Ca with variable response of the serum Ca. In the hyperparathyroids, renal P conservation compensated for the increased fecal loss, P balance being maintained. Unlike in normal subjects serum P remained at its initial level or fell more slowly. Increased fecal Ca produced more negative balances, though serum Ca was maintained. The hypoparathyroid patients responded uniquely in that fecal P excretion did not rise sufficiently to alter the balances. In one, P balance remained positive due to decreased urinary P output; serum P rose progressively from its initially high level. Concurrently, serum Ca level fell without subsequent rebound. The parallel changes in serum levels and urinary excretions of P suggest that the former is primary. Fecal K excretion increased and urinary K fell in all subjects.

8 *Biochemistry*

REMOVAL OF RADIOSTRONTIUM FROM MILK. Leon Singer and W. D. Armstrong, Dept. of Physiol. Chem., Univ. of Minn., Minneapolis, Minnesota.

Radiostrontium^{85} added to milk can be partially removed by contacting homogenized milk with bovine bone modified by preparatory treatment. Anorganic, potassium hydroxide-glycol ashed, and fat-free bone treated with 4N calcium chloride and 8 N sodium hydroxide have given the best results. Columns of bone (15 x 1.0 cm) containing 10 grams of 40-60 mesh modified bone with a flow rate of 10 ml/5 min have removed 45 percent of the added Sr^{85} from a quart of milk in a single pass. Increasing the column diameter to 1.8 cm, the flow rate to 10-15 ml/min, and the amount of anorganic bone to 25 grams and the fat-free treated bone to 33 grams resulted in removal of 57 and 75 percent of added Strontium^{85}, respectively. No alteration in Ca, P, Na and K composition of milk is seen with milk from the large column of anorganic bone although some reduction in calcium is seen with that obtained from the fat-free bone column. Addition of small amounts of calcium to the milk treated with fat-free bone is considered practical. A column may be regenerated for repeated trials by small volumes of the calcium and alkaline solutions. The effects of time of contact, temperature, solid-to-milk ratios and methods of mixing on removal of Sr^{85} in simple shaking experiments have been determined.

9 *Biochemistry*

THE DISCRIMINATION AGAINST STRONTIUM BY BONE GROWN IN VITRO. F. W. Lengemann (intr. by C. L. Comar.) Lab. of Radiation Biology, New York State Veterinary College, Ithaca.

In vitro culturing of embryonic bone showed that both rat and chick bone could discriminate against strontium in favor of calcium. The ends of the bones showed lower Sr^{85}/Ca^{45} ratios than the shafts. The selection appeared to be independent of the serum used for the medium or the age of the bones. Boiling the bones for 2 minutes in isotonic saline destroyed the ability to select against strontium. With live bone the selective mechanism functioned at 37° but not at 4° and 25°C. Selection occurred at some point after the initial entry into bone and was associated with the incorporation of the radioisotopes into a less-labile form of bone mineral. The Sr*/Ca* ratio as well as the total uptake of radioisotopes could be varied by incorporating inhibitors into the medium. (Supported by a grant from the U. S. Atomic Energy Commission AT-(40-1)-1829.)

10 *Biochemistry*

COMPARATIVE ULTRAFILTRATION OF Sr^{85} AND Ca^{45} FROM SERA OF NORMALS AND OF PATIENTS WITH PROTEIN AND CALCIUM ABNORMALITIES. Marianne Kara and Joseph Samachson (intr. by Thomas F. Gallagher.) Montefiore Hospital, New York.

In vitro ultrafiltration of Sr^{85} and Ca^{45} from normal human serum has confirmed that Sr filters to a greater extent than Ca. The average filterability of Sr^{85} was 65.9%, that of Ca^{45}, 54.5%, the average Sr^{85}/Ca^{45} ratio was 1.21. Ultrafiltration of Sr^{85} and Ca^{45} was carried out on sera containing abnormal proteins or protein distribution of 9 patients with Boeck's sarcoidosis and of 7 with multiple myeloma. The A/G ratio of sera of patients with Boeck's sarcoidosis ranged from 0.6 to 1.8 while that of patients with multiple myeloma ranged from 0.22 to 0.63, with the exception of one who had a ratio of 2.3. Despite these abnormalities in serum proteins, the ultrafilterability of Sr^{85} and Ca^{45} was similar to that of normals, the average Sr^{85}/Ca^{45} ratio being 1.23. Therefore, Ca is more completely bound than Sr to serum proteins even in patients with protein abnormalities. The ratio of ionized to protein bound Ca, the dissociation constant K, remained the same irrespective of whether the total serum protein is increased in the presence of normal or elevated serum calcium levels. (Supported by a grant from the U.S. Public Health Service.)

11 *Pathology*

A NEW HISTOCHEMICAL REACTION FOR DEMONSTRATING CALCIUM. R. Eisenstein, M. Werner and S. Papadiannis (intr. by George M. Hass). Dept. Path., Presbyterian-St. Luke's Hospital, Chicago, Illinois.

Sodium chloranilate is useful as a reagent for the quantitative analysis of calcium in solution, for under appropriate conditions it reacts with calcium to form an insoluble complex. Other inorganic ions in bodily fluids do not interfere. If tissue sections containing deposits of calcium are immersed in the reagent, a colored crystalline product appears in the areas of calcium deposition. The reaction appears to be as specific for calcium in the tissue as it is for calcium in solution. Various counterstains may be used and dehydration or permanent mounting of sections offer no problems because of the insolubility of the reaction product. This procedure has been used on a variety of tissues, including those of rabbits with hypervitaminosis D. It appears to be as sensitive as the more commonly used methods for demonstrating calcium in tissue section and is more specific.

12 *Pathology*

STUDIES OF CALCIUM METABOLISM OF PERFUSED EXTREMITIES OF DOGS. U. Barzel, S. Fell, J. Samachson and H. Spencer (intr. by G. Godman.) Montefiore Hospital, New York.

Calcium homeostasis is influenced by parathyroid activity and renal function. In order to study the effect of various agents known to affect bone directly, the perfusion technique of isolated limbs of dogs in which an extracorporeal pump and bubble oxygenator are employed was used (Creech et al). Prior to the testing of agents affecting bone metabolism, changes of plasma electrolytes during perfusion were studied. Oxygenated canine donor blood (95% O_2+5% CO_2) was perfused at an approximate rate of 100 ml/min. for 1 hour, occasionally for 2 hours. Ca, P, Na, K, Cl, CO_2-combining power and pH of blood circulating through the isolated limb and of the systemic circulation were frequently tested during perfusion. Except for an increase of plasma K and P, due to some hemolysis during perfusion, the concentration of all electrolytes did not change significantly. Hypocalcemia of the circulation of the isolated limb, induced by Na-EDTA, stimulated the release of calcium and of previously injected Ca^{45} and Sr^{85} from bone without implicating parathyroid action or renal function. The effect of parathyroid hormone and of corticosteroids on changes of calcium metabolism of the isolated limb will be discussed. (Supported by a grant from the U.S. Public Health Service.)

13 *Nutrition*

THE INFLUENCE OF AGING ON CALCIUM METABOLISM IN RATS. J. Kastelic, R. Hironaka*, and H. H. Draper. Division of Animal Nutrition, University of Illinois, Urbana.

Using Ca^{45} and balance techniques a study was undertaken to compare the efficiency of calcium absorption, the endogenous excretion and the turnover rate of plasma calcium in mature (10-12 months old) and aged (22-32 months old) rats. After administration of a single tracer dose of high specific activity calcium chloride, urine and feces collections were made daily for 18 days. The true absorption of dietary calcium (a mixture of $CaHPO_4$ and $CaCO_3$) was slightly greater ($P = < 0.05$) in aged than in mature rats (48.8 ± 9.2% vs. 38.0 ± 6.1%). The total endogenous calcium excretion of the aged animals was significantly higher ($P = < .01$) than that of the mature rats (15.93 ± 1.91 mg/day vs. 12.77 ± 2.28 mg/day). Most of the rats were in slightly positive balance during the experimental period. The apparent biological half-Life of plasma calcium, estimated from the decline in specific activity of fecal calcium during the post-absorptive state, was 7.73 ± 2.85 and 7.79 ± 3.17 days for the aged and mature groups, respectively ($P = > .05$).

14 *Nutrition*

UTILIZATION OF YOLK-SAC CALCIUM AND PHOSPHORUS BY THE NEWLY HATCHED CHICK. T. A. Crowley*, A. A. Kurnick*, M. G. Vavich*, and A. R. Kemmerer. Depts. of Poul. Sci. and Agri. Biochem., Univ. of Arizona, Tucson.

Utilization of yolk-sac Ca and P by newly hatched chicks between the 19th and 21st day of incubation was determined. Newly hatched chicks were counted at 6 hr. intervals. Half the chicks were removed from the incubator, weighed, sacrificed, and the yolk-sac removed. Yolk-sac and carcasses (entire chick except yolk-sac) were analyzed for Ca and P. Remaining chicks were left in the incubator until the end of the incubation period. Half the chicks removed from the incubator on the 22nd day were sacrificed and treated as described above. Remaining chicks were placed on a cerelose-blood fibrin diet containing .031% Ca and .06% P. Carcasses and yolk material of chicks hatched at various 6 hr. intervals contained similar Ca and P concentrations. Yolk-sac Ca:P of newly hatched chicks ranged from 2.43 to 2.95 mg., while that of corresponding carcasses from 1.27 to 1.37 mg. After 21 days of incubation, Ca:P of yolk-sac and carcasses ranged from 7.76 to 2.63 mg and 1.79 to 1.33 mg, respectively. This indicated a greater rate of P than Ca transfer from yolk to chick and a greater loss of P than Ca from chick to excreta. Survival on the deficient diet averaged 9 days. Analyses of dead chicks indicated a greater retention of P than Ca, opposite of that noted during incubation.

15 *Nutrition*

CARBOHYDRATES AND INTESTINAL ABSORPTION OF RADIOCALCIUM IN THE RAT. Oliver W. Vaughan* and L. J. Filer, Jr. Ross Laboratories, Columbus, Ohio.

This report is a summary of experiments designed to determine why lactose and several sugars that are not usually found in human or animal diets have the ability to increase the utilization of dietary calcium, while common dietary sugars, sucrose, glucose, and fructose, are ineffective. Ligated duodenal or ileal segments in anesthetized rats were injected with 0.5 ml of solution containing 2.5 mg of $CaCl_2$, 5 μc of $Ca^{45}Cl_2$ and 50 mg of the test sugar. Absorption of calcium was estimated by measuring the deposition of Ca^{45} in the femur. Neither lactose, sorbitol, glucose, sucrose, fructose, galactose or xylose significantly increased the rate of calcium absorption from the duodenum. However, each of these sugars markedly enhanced calcium absorption from the ileum. These findings indicate that lactose and certain other sugars owe their enhancing action on calcium utilization to the fact that they are slowly absorbed from the intestinal tract and are available for their role in calcium absorption throughout the small intestine. Mg^{++} and Sr^{++} are probably absorbed from the intestine through the same sugar-requiring mechanism. Mg^{++} and Sr^{++} decrease Ca^{++} absorption in the presence of glucose or lactose, $PO_4^{\equiv}$ decreases Ca^{++} absorption in the presence or absence of sugars and Na^{+} is without effect.

162. MINERAL METABOLISM– Magnesium

/ *Physiology*

MG 28 EXCHANGE BETWEEN BLOOD AND LUMBAR CSF IN MAN. J.H. Mendelson and B.A. Barnes (intr. by R.G. Grenell), Mass. Gen. Hosp. and Harv. Med. Sch., Boston, Mass.

Mg 28 was obtained from the nuclear reactor of the Brookhaven National Lab. with a S.A. of approximately 500 uc of Mg 28-Al 28 equilibrium mixture per 1 gram or less of stable Mg. At zero time Mg 28 was injected intravenously into healthy adult male volunteers. At 4 hour intervals samples of blood and lumbar CSF were obtained over a 24 hour period. All urine was collected to determine urinary loss of Mg 28. Aliquots of serum, CSF, and urine were counted in a gamma well counter. Stable magnesium was determined in all samples by a Titan yellow technique. At 4 hrs. a small but significant amount of Mg 28 had entered the lumbar CSF. The ratio of specific activities of serum and lumbar CSF reached unity at about 20 hours after injection. Furthermore, since the concentration of stable magnesium is greater in the CSF, the percent injected dose of Mg 28 in the CSF per unit volume was considerably higher than that of sera. These findings are consistent with a process of active transport which maintains a concentration gradient of exchangable Mg between serum and lumbar CSF. (Supported by a grant from the National Institute of Mental Health and Atomic Energy Commission.)

2 *Physiology*

THE EFFECT OF DIET ON THE TISSUE FLUORIDE:MAGNESIUM RATIO IN THE RAT. William C. Foster, W.A. Sterling*, J. P. Rush,* and J. A. Rehm.* Lab. of Physiology Research Philadelphia.

A study of the effect of the ingestion of microquantities of fluoride on the electrolytes in the tissues of rats was undertaken. Groups of ten albino rats were placed on eight diets; high and low magnesium, calcium, fluoride and lipid for sixty days ad libitum. After sacrifice, the fluoride content of the tissues was determined by a modification of the Singer-Armstrong method, whereas the tissue magnesium was determined by the method of Neill and Neely. The fluoride-magnesium ratio in soft tissues appeared to be more closely related to that of the mineralized tissues in rats receiving the high lipid diet with distilled water. In rats receiving the high lipid diet plus high magnesium and high fluoride, the fluoride:magnesium ratio increased in the soft tissues and decreased in the mineralized tissues. (Supported by a grant from the National Institute of Dental Research.)

3 *Pathology*

HISTOPATHOLOGY OF MAGNESIUM DEFICIENCY IN THE RAT. J.M.Craig/, K. W. Ko* and F.X. Fellers*. Children's Medical Center, Harvard, The Children's Cancer Research Foundation, Boston, Mass.

Magnesium deficiency in the rat causes disturbances in many tissues including skin connective tissues, teeth and kidneys. Forty male, 75 gm. Sprague-Dawley rats were divided into control and experimental groups of 16 and 24 each; 5 animals were sacrificed at weekly intervals. Chemical analyses of urine, serum, muscle and skeleton, and complete histological examinations were done with particular histochemical analyses of the kidneys. A degenerative lesion in the first part of the proximal tubule was found associated with histochemical changes. In addition, a second lesion characterized by the deposition of calcific material in the terminal portion of the second part of the proximal convoluted tubule was found which extended into the descending thin limb of Henle. In the colonic mucosa there was depletion of the mucous cells, and alterations in the size and staining reactions of the goblet cells. Degenerative changes were present in voluntary muscle of animals kept on the deficient diet for 5 weeks. (Supported by grants (C1975 and H2656) from the National Institutes of Health and Nephrosis Foundation.)

/Now at the University of Pittsburgh

4 *Nutrition*

THE EFFECT OF MAGNESIUM AND VITAMIN B_6 IN PROTECTING AGAINST RENAL OXALATE DEPOSITION IN RATS. S. N. Gershoff and S. B. Andrus. Harvard School of Public Health and Harvard Medical School, Boston, Mass.

This laboratory has described gross renal concretions of calcium oxalate in rats deficient in vitamin B_6. This study concerns the affects of different dietary levels of Mg and vitamin B_6 on the development of such concretions and on the production of diffuse renal oxalate deposition induced by ethylene glycol. In the first experiments 9 groups of rats were fed purified diets containing either 20, 40 or 400 mg.% of Mg and 0, 0.1 or 0.4 mg.% of vitamin B_6 for 6 weeks. Oxalate concretions were observed only in those animals receiving no B_6 and with the two lower Mg levels. The high level of Mg completely protected against oxalate depositions in B_6 deficient animals. In the second experiment 4 groups of rats were fed purified diets containing 40 or 400 mg.% of Mg and 0.2 or 20 mg.% of vitamin B_6 for 30 days. Drinking water was provided by a 0.25% solution of ethylene glycol. All of the rats receiving the low levels of B_6 and Mg showed renal oxalate deposition. Partial protection was obtained with the high vitamin B_6 diet and complete protection with the high Mg diets.

163. MINERAL METABOLISM– Potassium

/ *Physiology*

THE IN VITRO STEADY-STATE RELATIONSHIP BETWEEN INTERNAL AND EXTERNAL POTASSIUM OF THE RAT DIAPHRAGM. Norman G. Levinsky*, George W. Gorham* and Arnold S. Relman. Mass. Mem. Hosps. and Boston Univ. School of Med., Boston, Mass.

A simple in vitro technique has been developed for observing steady-state electrolyte concentrations in intact rat diaphragms in an oxygenated Krebs-Ringer medium. Intracellular concentrations change initially (K from 160 to 140 mEq/L; Na from 6 to 40; Cl from 0 to 14) but thereafter are constant for 12 hours. Diaphragms leached in K-free media rapidly reaccumulate K to "normal" in vitro levels at any time during this 12 hour period. Tissue K is unaffected by lowering external K from 9 to 1 mEq/L. Diaphragms from K-depleted animals with plasma K's of 2-3 mEq/L accumulate K when incubated at a bath K of 1 mEq/L. As bath K decreases from 0.8 to 0.06 mEq/L steady-state tissue K falls linearly from 36 ("normal") to 8 mEq/Kg dry weight. Extrapolation of this relationship suggests that about 7 mEq/Kg would remain in a K-free medium. These data indicate that in most in vivo K-depleted states, a fall in plasma K is not by itself sufficient to account for reduction in muscle K. The data also suggest that a significant fraction of muscle K may be relatively non-diffusible. (Supported by PHS Grant H-2767.)

2 *Biochemistry*

EFFECT OF EXTRACELLULAR ATP ON ERYTHROCYTE POTASSIUM TRANSPORT. Clarence S. Potter and John W. Rosevear (intr. by H. L. Mason). Mayo Clinic, Rochester, Minnesota.

Intracellular erythrocyte ATP has been frequently implicated as the primary factor in maintenance of the normal erythrocyte-plasma potassium concentration gradient. In the present experiments, extracellular ATP was found to oppose this normal gradient. Neutral sodium ATP added to heparinized human blood (9 mmoles ATP/l. blood) and incubated at 38° under 95% O_2 and 5% CO_2 caused plasma potassium to rise to 150-180% of control level in 2 hours. Since ATP cannot enter the erythrocyte except under acid conditions, it must be acting at the cell exterior. At equivalent concentrations AMP, inorganic pyrophosphate, inorganic phosphate, ITP and sodium chloride had no significant effect. ADP also caused a plasma potassium rise which was, however, slower and less than that caused by ATP. The effect of ATP on the erythrocyte potassium transport velocity constants k_1 (in) and k_2 (out) was evaluated by means of K^{42} exchange experiments and equations which were developed for conditions of changing plasma potassium concentration. ATP decreased k_1 and increased k_2, making nonspecific effects unlikely. Studies are continuing to determine whether extracellular ATP could be acting on the potassium transport mechanism in the same manner as intracellular ATP but in the opposite direction.

XUM

164. MINERAL METABOLISM–Sodium

1 *Physiology*

TISSUE ELECTROLYTES OF RATS GIVEN EXCESS SODIUM CHLORIDE. Arthur S. Haight and John M. Weller. Univ. of Michigan Med. School, Ann Arbor, Mich.

Hypertension has been reported to result from excess NaCl ingestion. Studies were done to determine tissue water and electrolytes of control rats and rats given excess NaCl. Rats ingested excess NaCl either as 2% NaCl drinking water or up to 8.4% NaCl in food for as long as 55 wks. With excess NaCl ingestion water content of skeletal and heart muscle usually decreased, with calculated cell water (based on extracellular Cl) consistently decreasing. Potassium content of skeletal and heart muscle usually decreased, whereas sodium increased only in rats receiving 2% NaCl in water. Aortas of all rats given excess NaCl showed increased sodium and potassium contents. Hypertension occurred in only one-third of rats on 2% NaCl in water and in none receiving excess NaCl in food. The striking electrolyte changes in hypertensive rats were: hypernatremia, increased skeletal and heart muscle sodium contents, and decreased skeletal and heart muscle potassium contents. It is concluded that excess NaCl ingestion does not consistently result in hypertension. Muscle electrolyte changes are more profound in hypertensive rats; aorta electrolyte changes are not.

165. MUSCLES

1 *Physiology*

ACTIVITY OF THE PARTURIENT RAT UTERUS, AS MEASURED BY THE "SUCROSE GAP" METHOD. J. M. Marshall, Johns Hopkins Medical School, Baltimore, Md.

It is possible to measure, with external electrodes, membrane resting and action potentials from a muscle strip if the short circuiting between electrodes is negligible. This is done by increasing the external resistance of the muscle between the electrodes by replacement of the interstitial fluid with sucrose. This method permits continuous recording of membrane activity for periods of several hours. The following observations were made: The membrane underwent rhythmical cycles of depolarization, associated with action potential discharge, and repolarization. In vitro application of progesterone abolished this activity. Application of oxytocin stimulated depolarization and action potential discharge. Ca-free solutions depolarized about 20 mv. and abolished action potentials. Addition of 0.25 mM Ca to the Ca-free solution increased the membrane potential slightly, and then oxytocin further increased it to a level where action potentials appeared. Isotonic KCl depolarized the membrane about 28 mv., whereas isotonic K_2SO_4 depolarized about 48 mv.

7 *Physiology*

AN ANALYSIS OF ALTERNATING MOVEMENTS OF THE HUMAN ARM. Robert W. Ramsey, A. H. Norris*, N. W. LeVora*, N. W. Shock, Sibyl Street*, John Bower*, M. R. Miller*, Rosser Rudolph* and Alfred Szumski*. Medical College of Virginia, Richmond, Virginia, and the Gerontology Branch, National Heart Institute, and the Baltimore City Hospitals, Baltimore, Maryland.

Fenn, W. O., J. Applied Physics 9, 165, 1938 showed that, if the torque is plotted against the displacement of a limb executing alternating (wagging) movements at maximal velocity, each individual has a characteristic pattern of torque-displacement curves for different amplitudes of swing. Fenn's records were limited to single swings because of limitations of instrumentation. The present analysis is based upon repetitive swings. There are minor differences in instrumentation and methodology used by the two laboratories but basically both recorded displacement, velocity and acceleration of alternating movements of the whole arm by suitable electrical transducers. Fenn's earlier work is confirmed, in particular the period of such swings increases linearly with the displacement. In addition, our results show action (energy x time) increases approximately linearly with increasing displacement. Also maximum momentum increases linearly with increase in the logarithm of the displacement except for swings of very small amplitude. Supported in part by a grant from N.I.H.

3 *Physiology*

EXPERIMENTAL MUSCULAR DYSTROPHY INDUCED IN RATS BY DIMETHYL-p-PHENYLENEDIAMINE. C. Jasmin and P. Bois*. School of Medicine, University of Montreal and University of Ottawa, Canada.

Investigations have been carried out on the toxic effects of some p-phenylenediamine derivatives in different species of animals. The dimethylamino derivative in a 1% saline solution injected subcutaneously into rats in a volume of 0.2 ml. twice daily causes acute degenerative lesions of voluntary muscles. The lesions are characterized by swelling and fragmentation of muscle fibers with loss of striation. There is also an increase in the number of sarcolemmal nuclei and proliferation of macrophages originating from the endomysial connective tissue. After 3 to 4 days of continuous treatment, regeneration occurs with formation of long nuclear chain (rowing) and of multinucleate plasmodial masses (muscle buds). These experimental lesions can be partly prevented by treatment with serotonin (1 mg. s.c., twice daily) and methylene blue (5 mg. s.c. daily). Histochemical enzyme studies reveal a reduction of succinic dehydrogenase and an increase in 5-nucleotidase activity. (Supported by grant from The Muscular Dystrophy Association of Canada).

4 *Nutrition*

CHARACTERISTIC MUSCLE AMINO ACIDS AND PEPTIDES IN INHERITED MUSCULAR DYSTROPHY OF THE CHICKEN. D.W. Peterson*, E.W. Martin, A.L. Lilyblade, J.B. Allred, and V.S. Asmundson. Dept. of Poultry Husbandry, University of California, Davis

A hereditary form of muscular dystrophy in the chicken has been described by Asmundson and Julian (J.Heredity 47:248 1956). Comparison of extracts of superior pectoral muscles of 7 month old dystrophic birds with those of normal birds revealed marked differences in certain ninhydrin reacting components. The major difference was a 24-fold increase in taurine, indicating a disturbance in sulfur metabolism. Dystrophic birds showed increases in alanine and decreases in carnosine and anserine. The following results are typical (values in mg/gm wet muscle; D = dystrophic; N = normal. Each is a mean of 4 birds): Taurine D = 2.10, N = 0.088; alanine D = 0.33, N = 0.14; carnosine D = 0.86, N = 2.96; anserine D = 4.03, N = 8.82. (Aided by NIH-B-1644).

166. MUSCLES–Chemical Properties

1 *Physiology*

THE QUANTITY OF CHEMICAL CHANGE IN MUSCLE ASSOCIATED WITH SHORTENING AND WORK. W. F. H. M. Mommaerts and Krikor Seraydarian. Univ. of California, Los Angeles.

In the iodoacetate-poisoned anaerobic frog sartorius muscle, breakdown of high-energy phosphate compounds is irreversible and expresses itself mainly as a formation of creatine from phosphocreatine. The total amount of phosphocreatine breakdown so measured has been determined by pair-wise analyses on muscles, one of which was the unstimulated control while the other performed 12 tetanic contractions of 0.4 seconds duration, under different loads causing various degrees of shortening. There was considerable scatter of the results, but nevertheless the conclusion emerged that definite amounts of phosphocreatine breakdown can be assigned to the activation, the shortening and the work performance of such muscles. (From the Los Angeles County Heart Association Cardiovascular Research Laboratory in the Department of Medicine)

167. MUSCLES–Contractile Proteins

7 *Physiology*

METABOLIC BUFFERING IN ACID-BASE IMBALANCE. Richard B. Tobin (intr. by Wallace O. Fenn.) Dept. of Physiology, Univ. of Rochester, Rochester, N.Y.

Since Wallace and Hastings (JBC 144:637,1942) and Tobin (AJP 186:131,1956) found no change of calculated intracellular pH in acidotic cats, a study of some intracellular mechanisms which might modify acidosis has been carried out. Adult rats were made acidotic by intraperitoneal injection of 0.15N HCl and alkalotic by injection of 0.15 M $NaHCO_3$. Volume loads of 4% of the body weight were used. Three hours later samples of muscle, blood, and liver were analyzed for lactic acid and fractions of acid soluble phosphates. Acidotic animals showed no change in lactate or phosphocreatine in muscle, but a fall in muscle inorganic phosphate. The blood and liver showed a rise in inorganic phosphate. Alkalotic animals showed a rise of lactate and inorganic phosphate in muscle, a fall of inorganic phosphate and an increase in lactate in blood, and a rise of lactate plus phosphate in liver. It is felt that breakdown of phosphocreatine is not a mechanism of cellular buffering. Lactate retention moderates alkalosis but not acidosis.

3 *Physiology*

TITRATION CURVES OF FROG MUSCLE IN VIVO. Hubert R. Catchpole, Milton B. Engel* and Norman R. Joseph. Depts. of Path., Orthodontics and Chem., Chicago Prof. Colleges, Chicago, Ill.

Colloidal surface properties of frog muscle were studied in vivo before and after modifying the respiration in 2 ways: in one, by temperatures of 25° and 2° C; in the other, by the use of respiratory inhibitors and metabolites. The thigh muscles were exposed, lightly scraped, and the colloidal charge density of the surface determined at a series of pH's between 7.4 and 2.2 (Arch. Biochem. & Biophys., 84: 224, 1959; 85: 209, 1959). This is equivalent to a titration curve of the surface. This curve was essentially independent of the temperature, indicating that although energy production was greatly lowered, the electrolyte distribution was unchanged. After exposure of the surface to 2, 4 dinitrophenol, iodoacetate, lactate, cyanide and bicarbonate ion, the titration curves were modified. Respiratory change without change in colloidal state therefore need not affect ion distribution. Inhibitors and metabolites which decrease respiration are bound to the colloidal matrix, thereby altering its properties and producing redistributions of all other anions and cations. (Supported by a grant from the USPHS, Grant No. A968C3).

4 *Biochemistry*

THE ISOLATION AND SYNTHESIS OF CARNITINE. Max Jellinek* and D. R. Strength. Dept. of Biochemistry, St. Louis Univ. Sch. of Med., St. Louis, Mo.

Simplified procedures were developed for isolation and synthesis of carnitine. Reineckates, soluble in water at pH 8 and insoluble at pH 2, were prepared from concentrates extracted from Difco Beef Extract with aqueous methanol. The reineckates were recrystallized three times from aqueous acetone. Carnitine was obtained from the purified reineckate by displacement with silver ion. The dried product was dissolved in methanol and crystallized after addition of isopropanol. After recrystallizing twice the product melted 135-137°. Anal. $C_7H_{16}NO_3Cl$. Calcd.: C 42.52, H 8.16, Cl 17.93; Found: C 42.10, H 8.36, Cl 17.65. DL-carnitine was synthesized by the reaction of trimethylammonium chloride and epichlorohydrin in methanol to form (3-chloro-2-hydroxypropyl) trimethylammonium chloride (m.p. 193-194°). This was converted to the nitrile with NaCN in water and the nitrile (m.p. 231-232°) hydrolyzed with HCl to DL-carnitine. After recrystallizing from methanol-isopropanol the product melted 197-199°. Anal. Found: C 42.74, H 8.25, Cl 18.22.

1 *Physiology*

PROPERTIES OF TWO FRACTIONS OF MYOSIN A ISOLATED BY CHROMATOGRAPHY. J. Brahms (intr. by M. F. Morales.) Department of Biochemistry, Dartmouth Medical School, Hanover, N. H.

It has been reported that by application of chromatography on DEAE cellulose, myosin A freed of myosin B is resolved into at least two components, α and β (Brahms, J.A.C.S. 81:4997 1959). Further studies suggest reversible transformation of α to β-component. The transformation of β into α is demonstrated in experiments in which the β was submitted to a rechromatography on DEAE cellulose; it yielded a major α-component. Other observations suggest that β may be transformable into α by aging or by warming briefly to 25° C. The transformation of α to β-component was observed when α was heated briefly to 25° C in the presence of ATP and Ca^{++} or Mg^{++}; the rechromatography of this solution yielded an α and a β-component. The formation of β can also occur at 0° C if to the previous system PCMB is added in amount equal to the 1/2 value of SH groups of myosin. Thus, purified α and β-components have the same Mw, but different length, as demonstrated by light scattering measurements; the β is more extended; also its specific ATPase activity is greater. The results of these investigations suggest that myosin molecules can exist in two forms of different length. (This work was done during tenure of a Rockefeller Fellowship, and was supported by U.S.P.H.S. Training Grant 2G-174.)

2 *Physiology*

STUDIES ON MYOSIN OF FROG'S SARTORIUS AT REST, DURING CONTRACTION AND IN FATIGUE. Maria W. Seraydarian and Ella B. Williams*. Zoology Dept., UCLA, Los Angeles, Calif.

A lowering of solubility of muscle proteins as a result of activity has been studied by Dubuisson in rabbit skeletal muscle. To further investigate this phenomenon, frog's sartorius was chosen. The muscle was quick-frozen either at the peak of a short tetanus or in fatigue, simultaneously with the symmetrical muscle of the pair kept at rest, as a control. The frozen muscles were ground in liquid nitrogen and myosin was extracted with Guba-Straub solution. No difference in the extractability of proteins was observed and the yield of myosin in each case was approximately 20 mg per 1 gm muscle. Furthermore, the ATPase activity of the preparation, the concentration of sulfhydryl groups and phosphoryl groups did not reveal any change. The ATP concentration in the muscle in either case was the same, while creatinephosphate was reduced to 50% in fatigue. These findings do not exclude the possibility of changes in the myosin in situ, but no changes were observed in the extracted protein. (This work was done during the tenure of an Established Investigatorship of the Los Angeles County Heart Association by M.W.S.).

3 *Physiology*

ACCELERATING EFFECT OF H MEROMYOSIN ON THE POLYMERIZATION OF ACTIN. A. Martonosi, (intr. by J. Gross), Cardiac Biochemistry Research Lab., Depts. of Medicine, Mass. Gen. Hosp. and Harvard Medical School, Boston, Mass.

If H meromyosin (HM) (0.4 mg/ml) is added to a dilute actin solution (0.1 mg/ml) in 0.1 M KCl there is an instantaneous increase in the intensity of the scattered light, with a final value equal to that obtained on adding HM to an 0.1 mg/ml F actin solution. Subsequent addition of ATP reduces the scattering intensity to the same level in both cases. Thus it appears that HM promotes the polymerization of actin. When the rate of KCl induced polymerization is measured in a more concentrated actin solution (1 mg/ml) containing varying amounts of HM (0 - 0.12 mg/ml) an initial increase in scattering roughly proportional to the HM concentration is observed, followed by a linear increase, the rate of which is independent of the HM concentration. These results indicate that the polymerization promoting effect of HM is not catalytic. The effect of myosin on actin polymerization has been considered by Szent-Györgyi and by Laki and Clark (cf Arch. Biochem. 30, 187, 1951) to be catalytic. The accelerating effect cannot solely be due to the ATPase activity of HM, since other ATP splitting systems (Hexokinase-glucose, Cr-Crkinase) inhibit polymerization, as does pretreatment with HM in 0.6 M KCl or in the absence of KCl.

XUM

4

Physiology

A NEW MODEL OF THE STRUCTURE OF MYOSIN AND ACTIN FIBERS, OF THE ULTRASTRUCTURE OF THE SARCOMERE, AND THE MECHANISM OF MUSCULAR RELAXATION. Melvin D. Stern (intr. by S. Krop.) Ethicon, Inc., Somerville, New Jersey

A model of the structure of myosin and actin fibers is proposed in which the essentially cylindrical fibers are formed from a thin ribbon composed of myosin(or actin) macromolecules laterally aggregated in a staggered arrangement. The ribbon is twisted around to form an overlapped, helical band; the overlap dimension being equal to the 410Å striation spacing of myosin fibers or 1/4 the length of the myosin macromolecule. The resulting hollow, cylindrical fiber may expand without change in fiber length by a change in fiber striation spacing (or overlap dimension) concomitant with change in fiber diameter. Using these models, the Huxley-Hanson model of the Sarcomere is reexamined and reinterpreted to give a Sarcomere unit in which a myosin fiber is surrounded by three helical, concentric actin fibers connected to the Z membrane. The expansion of the myosin fiber relative to the actin fibers as a result of an ATP linked charging process results in muscular contraction; the elimination of the charge and radial contraction of the myosin resulting in relaxation of the muscle. Other mechanisms of contraction and relaxation are also discussed.

5

Physiology

PREPARATION AND PURIFICATION OF EMBRYONIC CHICK MYOSIN. David S. Love. (intr. by E. G. Boettiger.) Inst. Cell. Biol., Dept. of Zool., U. of Conn., Storrs, Conn.

A survey of the methods of preparing myosin from adult muscle was made in order to obtain this protein from chick embryos and newly hatched chicks as enzymatically active and homogeneous as possible. Although none of the methods per se employed in the adult muscle extraction met these requirements when applied to embryonic muscle, it was found that the use of $K_4P_2O_7$ in the presence of guanidine and cysteine resulted in reasonably high yields of enzymatically active protein. Electrophoretically homogeneous fractions were obtained only after purification by ultracentrifugation, $(NH_4)_2SO_4$ fractionation and DEAE-cellulose chromatography. The precipitate obtained between 35% and 50% $(NH_4)_2SO_4$ saturation was found to consist of four fractions by cellulose chromatography. Three of these fractions showed Ca activated ATPase activity and ATP sensitivity upon combination with actin, absorbed maximally at 280 mu, and salted out at 40-42% $(NH_4)_2SO_4$ saturation. The fourth fraction showed no enzymatic activity, absorbed maximally at 260 mu, and was soluble in saturated $(NH_4)_2SO_4$ and 10% TCA. All four fractions were found to be present in all embryonic stages used but their relative quantities varied. This variation can be correlated with the ontogeny of a functional endocrine system. (Supported by USPHS grant B-549.)

6

Physiology

STUDIES ON THE MYOSIN CREATINE KINASE SYSTEM.
Jean Botts and M. Judith Stone.* Dept. of Biochem., Dartmouth Medical School, Hanover, N. H.

A comparison is made of the ATP-ase activity of myosin when the substrate ATP is supplied directly and when ATP is formed and maintained through the action of creatine kinase on creatine phosphate and ADP. "Myosin A" and "myosin B" are prepared by extracting rabbit muscle mince at pH 6.7 for two hours in a KCl-ATP-Mg^{++} medium of ionic strength 0.5 M. "Myosin B" is separated from "myosin A" by stepwise decrease in ionic strength. Each myosin preparation then undergoes a 4- or 5-cycle purification. Results indicate that in 0.17 M KCl with Mg^{++} present the myosin ATP-ase activity is lowered to a greater extent in the two-enzyme system than in the corresponding one-enzyme system at the same ATP concentration. A possible interpretation of these results is that there is an interaction between the two enzymes. (Supported by a grant from the National Heart Institute.)

7

Physiology

REACTIONS OF ACTIN-ASSOCIATED NUCLEOTIDE. Teru Hayashi and Kenneth K. Tsuboi. Columbia U., N.Y. Hosp. and MBL, Woods Hole

The demonstrated availability of the actin-associated nucleotide for enzymatic phosphate turnover (Strohman, Fed. Proc. 17; 157, 1958) raised the question "Does the enzyme attack a protein-bound nucleotide, or a dissociated nucleotide?" A direct approach was not possible, but by use of ATP labeled in the two terminal positions, chromatography, and Dowex-1, which quantitatively removes free nucleotide from solution, conditions for the separation of nucleotide from F- (F-ADP) and G-actin (G-ATP) were determined. Consecutive transformations from F- to G- to F- gave a constant associated nucleotide. F-ADP depolymerized in the presence of A-P-P-P* formed G-A-P-P-P*. Likewise, G-ATP incubated in added A-P-P*-P* yielded G-A-P-P*-P*, which polymerized to form F-A-P-P*. These results confirmed the C^{14}-labeled ATP experiments (Martonosi, Gouvea, and Gergely, Abs. Biophys. Soc. 1959) and permits the conclusion that free ATP can replace (displace?) nucleotide from either G- or F-actin depolymerized. Paradoxically, prolonged Dowex-1 treatment does not touch the ATP of G-actin, indicating a firm binding. Thus, both possibilities remain open, and either would explain the finding that muscle powder treated with H_2O + Dowex-1 results in a non-polymerizable extract. (Supported by grants from MDAA and NSF to T. H.)

8

Biochemistry

SOME PROPERTIES OF AET-TREATED MYOSIN B ATPASE. Ken Hotta* and Manuel Morales. Department of Biochemistry, Dartmouth Medical School, Hanover, N. H.

Some properties of the ATPase activity of myosin B treated with s-β-aminoethylisothiuronium ("AET") have been studied. It has been found that the strong activation due to AET-treatment reported earlier (Morales et al., 1957; Morales and Durant, 1958) is accompanied by about a 50% reduction in the number of PCMB titratable SH groups. Especially in tetramethylammonium chloride solvents the ATPase activity of myosin B is simplified by AET-treatment; the effects of Ca^{++} and Mg^{++} ions are clearer, and the pH-dependence of the activity resembles that of a single group ionizing with a pK of ca. 6.8. This is the same apparent pK as for the ITP-ase activity of untreated myosin B, much as would be expected from the Blum-Gilmour-Koshland hypothesis of NTPase hydrolysis. The apparent pK at 6.8 may mean that after initial complexing some bound phosphate must be ionized fully before being further processed by the enzyme. The Van't Hoff plots of the V_m of AET-treated myosin B ATPase show no definite "breaks" in the temperature range 0° C to 25° C. (This work was supported by National Heart Institute Grant H-3598.)

9

Biochemistry

THE DEGRADATION AND REACTIVITY OF MYOSIN IN UREA SOLUTIONS. D. B. Wetlaufer. Harvard Univ. Biol. Labs. and Children's Cancer Res. Found., Boston 15, Mass.

While the limited proteolysis of myosin to yield more or less well-defined fragments has given several interesting results, this approach has the inherent limitation of an undefined relationship between the parent molecule and the digestion products. We have therefore begun to study rabbit skeletal myosin by means of selective and controlled degradations, beginning with solution in 8M urea, which disrupts secondary but not covalent bonds, followed by reduction specific for -S-S-, and oxidation specific for thiols. Double-sector cell sedimentation velocity(sed. vel.) studies of myosin in 8M urea resolve three myosin-urea fragments (MUF) of differing molecular size. This means that myosin contains at least three dissimilar peptide chains. The largest, MUF-3, comprises 3/4 of the total protein, while MUF-1 and MUF-2 each contribute 1/8. Viscosity and sed. vel. expts. show no time-dependence for the disruption. The sed. vel. pattern is unchanged when myosin is pre-treated with -SH blocking agents, or with NaSH. Lack of response to NaSH suggests the absence of interchain -S-S- bridges in myosin. Ferricyanide-oxidized myosin shows a more complex sed. vel. pattern but no change in MUF-1, suggesting the absence of -SH from this fragment. (Supported in part by a Research Fellowship of the American Heart Ass'n, 1956-58.)

10 *Biochemistry*

THE PARTICIPATION OF CALCIUM IONS IN THE RELAXING FACTOR SYSTEM (RFS) OF MUSCLE. J. Gergely and C. J. Parker, Jr.,* Mass. Gen. Hosp. and Harvard Med. School, Boston, Mass.

Parker and Gergely have recently obtained (Abstr. 135th ACS Meeting 7C, 1959) a granule (G) free "relaxing substance" (RS) from muscle. We have investigated the question whether the inhibition by Ca^{++} of the RFS depends on (a) the suppression of the production of the RS, (b) the inhibition of the RS, or, as suggested by various authors (Bozler, A. Weber, S. Ebashi), (c) the restoration of myofibril-bound Ca^{++} removed by the RFS. The action of the RS is inhibited if $(Ca^{++})>5x10^{-6}$ M. No clear answer to (a) has been obtained. Inhibition of the complete RFS requires larger concentrations of Ca^{++}, roughly proportional to the concentration of G. This is consistent with (c). Our studies on the removal of myofibril-bound Ca^{++} by G and Mg^{++} however do not support (c). About 50% of the bound Ca^{++} is removed, the same amount as by Mg^{++} alone. Ca^{++} depleted fibrils show normal ATPase activity and synaeresis after removal of G. Experiments with Ca^{45} show that binding of trace impurities of Ca^{++}, after removal of G, does not account for this. These experiments tend to support the view that although granules combine with Ca^{++} the action of the RFS depends on the interaction of the RS with myofibrils not involving the removal of bound Ca^{++}. (Supported by grants from the US PHS, the Musc. Dystr. Ass., and the Life Ins. Med. Res. Fund)

11 *Pharmacology*

THE CONTRACTILE PROPERTIES OF ACTOMYOSIN FROM RYANODINE AND IODOACETIC ACID POISONED RABBITS. Leonard Procita. Dept. of Pharmacology, State Univ. of New York Medical Ctr. Brooklyn 3, N.Y.

Utilizing the extraction proceedures of Szent-Gyorgyi actomyosin was obtained from muscles of rabbits rigored by the i.v. injection of 0.5-1.0mgm./kg. of ryanodine or 50-100 mgm./kg. of iodoacetic acid, and from rabbits anesthetized with thiopental. The contractile properties of the actomyosin were studied utilizing a method adapted from that of Hayashi and Rosenbleuth (J.Gen. Physiol. 36:139, 1952). The compressed fibers were suspended isometrically in a suitable medium and allowed to develop tension in the presence of ATP(5×10^{-3}M) over a period of 30 minutes. The total tension developed by actomyosin fibers from ryanodine and IAA rigored muscle was 45-65mgm. and 20-30mgm. respectively. The tension developed by actomyosin fibers from the relaxed muscle of thiopental anesthetized animals was of the order of 30-40mgm. The results show that the mechanical properties of actomyosin from rigored muscle depends upon the agent used to produce the rigor.
(Supported by USPHS Grant RG-5557)

168. MUSCLES–Contraction

1 *Physiology*

LOCAL ACTIVATION AND STRUCTURE OF SLOW STRIATED MUSCLE FIBERS OF THE FROG. Lee D. Peachey and Andrew F. Huxley (intr. by Frank Brink, Jr.) The Rockefeller Institute, New York and the Physiological Laboratory, Cambridge, England.

Light and electron microscopic examination of slow muscle fibers of the frog showed large irregular masses of contractile filaments and a sparce sarcoplasmic reticulum. In order to see if there was a correlated physiological difference from twitch fibers, single slow muscle fibers were isolated from tonus bundles of iliofibulari of Rana temporaria and stimulated opposite various bands with long (2-20 sec.) depolarizing d.c. pulses through 1.2-1.5μ diameter glass pipettes. Microscopic observation and cine records were made using interference optics. In contrast to twitch fibers of the frog where sensitivity is localized in the I-bands and activation spreads radially inward as far as 10μ without affecting longitudinally adjacent sarcomeres only 3μ away, the slow fibers have sensitive spots in both A-bands and I-bands. Furthermore, activation spreads as far longitudinally as radially, often affecting three I-bands. The strength of the response in each half I-band decreased with increasing distance from the pipette, sometimes resulting in asymmetric contraction of an I-band with the Z-line being pulled to one side. These results agree with the hypothesis, suggested previously by several authors, that intracellular impulse conduction in muscle takes place along specific pathways formed by certain elements of the sarcoplasmic reticulum.

2 *Physiology*

THE BIOSYNTHESIS OF A MUSCLE RELAXING AGENT. F. Norman Briggs and Franklin Fuchs*, Tufts Univ. School of Med., Boston, Mass.

The incubation of muscle microsomes with ATP and appropriate salts eliminates the usual delay of the inhibition of tension generation by a glycerinated muscle fiber following the direct addition of microsomes. High speed centrifugation of the incubation mixture (104,000 g for 1 hr.) demonstrates that during the incubation a soluble and dialyzable substance capable of inhibiting tension generation without delay is present. Analysis of the conditions required for the demonstration of this agent suggest that the agent did not preexist in the microsome but that the agent is actually synthesized during the incubation. The lack of correlation between the relaxing activity of the supernatant and quantity of microsomes used and the ability of the microsomes to form an active supernatant on successive additions of ATP argue against the notion of a simple leak of a pre-existing agent from the microsome. The evidence also suggests that ATP may be a substrate for the formation of the relaxing agent.

3 *Physiology*

EFFECT OF GALLAMINE (FLAXEDIL) AND ACETYLCHOLINE ON MOTILITY IN ANEURAL SMOOTH MUSCLE. John Ferguson. Creighton Univ. School of Medicine, Omaha, Nebraska.

The acetylcholine-blocking action of gallamine triethiodide was studied by using strips of aneural smooth muscle (chick amnion). Each of these was suspended in oxygenated Sollmann-Rademaekers' solution. Motility was recorded before and after the application of a drug. Gallamine in 10^{-7} to 5×10^{-4} was added to the bath in which each of 24 rhythmic strips was suspended. A concentration of 5×10^{-4} increased slightly spontaneous motility in the preparations. No inhibitory effects were noted. After gallamine the muscle reacted to stretch and release by enhanced activity as does normal amniotic muscle. Acetylcholine chloride in 2.5×10^{-7} augmented motility in amniotic strips. In one group of experiments, gallamine in concentration of 2.5×10^{-4} slightly diminished the subsequent excitant effect of acetylcholine. But, 1.5×10^{-4} gallamine added to the bath after acetylcholine depressed the acetylcholine-induced activity. In another group of experiments in which 24 preparations were used, the response to acetylcholine in the same muscle before and after gallamine was determined. Following the application of a drug, the muscle was washed and retreated. Large doses of gallamine slightly lessened the effectiveness of acetylcholine as was found in the preceding determinations. (Aided by Neb.Heart Assoc.)

4 *Physiology*

EVIDENCE FOR INCREASED SODIUM PERMEABILITY DURING ACTIVITY IN MAMMALIAN SMOOTH MUSCLE. C.Y. Kao, F. Bronner and D. Zakim*, Dept. of Pharmacology, State Univ. of N.Y., Downstate Medical Center, and Hospital for Special Surgery, New York.

Despite observations that "spike" activity in some mammalian smooth muscles persisted in media containing as low as 2-7% of the normal Na concentration, some similarities of ionic permeabilities in them with those found in nerve, skeletal and heart muscles have been shown by tracer experiments. Strips of isolated estrogen-dominated myometrium of rabbits were loaded with $Na^{22}Cl^{36}$ in Krebs' solution. During loading, some were unstimulated; and some, stimulated with 60 cycle AC, 3-4 v/cm, for 4 sec. every minute to assure end-to-end excitation. Upon elution into ordinary Krebs', Na^{22} from unstimulated muscles washed out quickly, only 0.1% of the total Na^{22} remaining in the muscles at 1 hr. In stimulated muscles, however, 6-8% still remained at 1 hr., disappearing thereafter with a rate constant of 0.14 hr^{-1}. Simultaneous determinations of Cl^{36} elution showed no significant difference between stimulated and unstimulated muscles; but in both, 2-4% of the total Cl^{36} remained at 1 hr. These preliminary results indicate that myometrial cells are more permeant to Cl^- than to Na^+, and that, as in other animal excitable tissues, permeability to Na^+ can be markedly increased by effective stimulation.
(Supported in part by USPHS grant A-1713).

XUM

5 *Physiology*

ISOTONIC VELOCITY TRANSIENTS IN MUSCLE AND THEIR RELATION TO THE CONTRACTILE MECHANISM. R. J. Podolsky (intr. by D. E. Goldman.) Naval Medical Research Institute, Bethesda, Md.

Whether the force in contracting muscle arises from (I) the elasticity of a single filament or (II) the mechanical interaction of two filaments is not clear. Because it bears on this question, the "quick release" phenomenon (the loss of tension accompanying short but rapid contraction) was re-examined. The tension on frog sartorius muscle was changed suddenly from P_o to $P < P_o$ and clamped at the final value. The change in length during and following the tension step was measured. After the tension step, the shortening velocity, v, ultimately reached the steady value expected from the classical force-velocity curve. However, at 0°C, v did not settle down to the steady value until at least 15 msec after P had been established. In this interval, v was first greater than, and then less than, the final steady value. The time course of this isotonic velocity transient, originating in the contractile mechanism, depended on the size of the tension step. Thus the shortening rate of the contractile element is not solely a function of the tension on the muscle; after a quick change in tension there are alternations in speed, which resemble "hunting" motions, as though the final steady speed were controlled by a feedback mechanism. Such a feedback mechanism would reconcile the loss of tension in a "quick release" with (I).

6 *Physiology*

RECOVERY OF CAROTID ARTERY STRIPS FROM COLD STORAGE. Lloyd Barr, David F. Bohr and Verle Headings. (Intr. by C. R. Brassfield.) Physiology Dept. Univ. of Michigan, Ann Arbor.

Dog carotid artery preparations stored at 4°C gained Na, lost K and their ability to respond to stimuli. After storage for 36 to 50 hrs, the responsiveness of helical strips, recorded isotonically, was tested by electric shocks every fifteen minutes throughout a recovery period in physiological solutions at 37°C. Sodium and potassium content of rings from the same arteries was measured after similar treatment. The rings were stimulated electrically in the same manner as the strips used for mechanical recording. Four recovery solutions were used containing respectively 3, 6, 12 and 24 mM K added as the Cl. Sucrose concentrations of the solutions were varied to maintain constant osmolarity. Sucrose was used for ECF determination. Intracellular K concentration rose during recovery from cold storage values (7 to 32 mEq/l) to steady state values of 55, 63, 76, 85 mEq/l for the 3, 6, 12, and 24 mM K recovery solutions respectively. The return of maximal responsiveness in the strips was roughly parallel to the attainment of the steady state K concentration. The strips also developed a contrature which was larger and occurred earlier as the K concentration of the recovery solution was greater. (Supported by a Michigan Heart Association grant.

7 *Physiology*

THE EFFECTS OF THYROID HORMONE AND L-EPINEPHRINE ON KINETICS OF CONTRACTION AND RELAXATION OF DOG SKELETAL MUSCLE. James P. Isaacs, Patricia F. Osgood, and William R. Brewster, Jr. (intr. by D.F. Proctor.) Johns Hopkins Univ. School of Med., Baltimore, Md., Univ. of Florida College of Med., Gainesville, Fla., and Anesth. Lab., Harvard Med. School, Mass. Gen. Hosp., Boston, Mass.

Studies have been made of the rates of contractin and relaxation of the supramaximally stimulated gastrocnemiusand plantaris muscles of athyroid, euthyroid, and hyperthyroid dogs. The animals were studied prior to and during the maintenance of a total sympathetic ganglionic block and during the infusion of l-epinephrine at rates of 0.25-8.0 micrograms per kilogram body weight per minute.

The rates of contraction and the tension developed were not affected by varying l-epinephrine or thyroid hormone concentration. The rate of relaxation, however, was increased by raising the concentration of either hormone. The rate of relaxation was decreased by blocking or reducing the concentration of either hormone.

Each hormone accentuated the effect of the other and had little effect in the absence of the other.

8 *Physiology*

EFFECT OF ISCHEMIA UPON THE KINETICS OF CONTRACTION AND RELAXATION OF DOG SKELETAL MUSCLE. William R. Brewster, Jr., James P. Isaacs, and Patricia F. Osgood (intr. by L. R. Dragstedt.) Univ. of Fla. Coll. of Med., Gainesville and Anes. Lab. of Harvard Med. Sch., Mass. Gen. Hosp., Boston.

The kinetics of isometric contraction and relaxation of skeletal muscles were observed in 34 dogs prior to and during ischemia. In 9 dogs, serum Ca^{++} was elevated to 18-30 mg % by i.v. $CaCl_2$. Contraction was produced by supramaximal nerve or direct muscle stimuli. In all dogs, ischemia initially slowed the rate of relaxation without change either in the rate of contraction or tension developed. Prolonged ischemia produced a progressive decrease in the rate of relaxation without change in the rate of contraction. With normal serum Ca^{++}, ischemia resulted in a progressive decrease and ultimate failure of tension development followed by a slow spontaneous development of tension during rigor. With an elevated serum Ca^{++} concentration, there was an increase in tension development during ischemia associated not only with a decrease in the extent of relaxation, but with terminal failure of relaxation and tetanus tension development. The data support the concept that relaxation is an active process integrally related to the rate and integrity of intermediary metabolism. Work supported by Air Force Contract #49-638.

9 *Biochemistry*

THE PARTICIPATION OF CHARGE TRANSFER COMPLEXES IN SMOOTH MUSCLE CONTRACTION. Benjamin Kaminer (intro. by Albert Szent-Gyorgyi) Institute for Muscle Research at the M.B.L., Woods Hole, Mass.

Charge transfer complexes between electron donors and acceptors of biological importance, can occur, as for example, in the case of serotonin (5HT) and riboflavine-5-phosphate (FMN)(Isenberg, I. & Szent-Györgyi, A.). Chemical and quantum mechanical evidence (Szent-Györgyi, A.; Pullman, A. & B.; Karreman, G.) both show that sevron blue and acridine red are electron acceptors. To test whether electron transfer is concerned in some way in muscular contraction, experiments were performed on a smooth muscle preparation. It will be demonstrated that 5HT, in combination with either FMN, sevron blue or acridine red, causes contraction of the isolated proboscis of Phascolosoma. The proboscis, stripped of its nerve and immersed in sea water for 1/2 - 3 hrs. does not contract spontaneously. 5HT (10^{-8} - 10^{-4} M) fails to excite this preparation. If, however, the proboscis soaks in either FMN, sevron blue or acridine red (10^{-4} M in sea water), 5HT will induce contractions. Sevron blue or acridine red may evoke rhythmic contractions; in such cases 5HT augments the force. These findings suggest that charge transfer complexes may play a role in the contraction of smooth muscle. (This work was done during the tenure of a Rockefeller Fellowship).

10 *Pharmacology*

EFFECT OF CAFFEINE ON RADIOCALCIUM MOVEMENT IN FROG SARTORIUS MUSCLE. C. P. Bianchi (intr. by A. M. Shanes.) Natl. Insts. of Health, Bethesda, Md.

Experiments by Axelsson and Thesleff (Acta physiol. scand. 44:55-66, 1958) suggest that caffeine initiates a process in the muscle membrane which leads to muscular contracture. The caffeine effect was found to occur without membrane depolarization and to be unaffected by either increasing or completely removing calcium by addition of EDTA to zero Ca Ringer's. In order to determine if caffeine was affecting membrane calcium which was not removed by EDTA (Gilbert and Fenn, J. Gen. Physiol. 40:393-408, 1957) measurements were made of radiocalcium influx and outflux as affected by caffeine. Resting radiocalcium influx in frog sartorii is increased 3-fold in Ringer's solution and in .080 M KCl Ringer's solution by .005 M caffeine. The increased influx in K^+ depolarized muscles is maintained. In the absence of caffeine radiocalcium influx increases only during the initial K^+ depolarization and returns to a resting level during maintained depolarization. Outflux is also markedly increased by caffeine in Ringer's solution in K^+ depolarized muscles, and in muscles which have been washed out for 120 minutes in zero Ca Ringer + .004 M EDTA. These results suggest that an increase in activity of membrane calcium underlies the membrane process initiated by caffeine leading to muscle contraction.

11

Pharmacology

CONTRACTION OF FROG MUSCLES INDUCED BY SMALL INCREASES IN POTASSIUM. C. P. Kratz, Jefferson Medical College, Philadelphia, Pa.

The rectus abdominis muscle of the frog maintained in a constantly flowing bath of bicarbonate-buffered Ringer's solution manifests a slow contractile response to potassium increases of from 4 to 8 millimolar (without removal of sodium). At the lowest level, contractions start in from 2 to 3 hours after the beginning of exposure, higher levels evoking a progressively more rapid onset. The maximum load lifted is of the order of 4 Gm., with practically all muscles capable of raising 3 Gm.; the maximum extent of shortening or tension developed by a given muscle is less than its maximum response to acetylcholine. The onset of contraction is accelerated by pretreatment of a muscle with low concentrations of cocaine or of ouabain; the potassium effect is inhibited quantitatively by calcium, while tubocurarine has no inhibitory action. The iliofibularis and semitendinosus muscles of the frog, less rich in small-nerve innervated fibers than the rectus, give similar but smaller potassium responses, while the sartorius, which consists only of twitch fibers, does not shorten.

12

Pharmacology

EFFECT OF INACTIVITY OF MUSCLE ON ELECTROMYOGRAPHIC SIGNS IN EXPERIMENTAL MUSCULAR DYSTROPHY. J. J. Fudema*, Y. T. Oester and A. J. Gatz*. Dept. of Pharmacology and Therapeutics, Grad. School and Stritch School of Med. of Loyola Univ., Chicago, and Dept. of Microscopic Anat., Med. Coll. of Georgia, Augusta, Ga.

Pappenheimer and Goettsch (Proc. Soc. 43:313, 1940) reported that denervated skeletal muscles of vitamin E deficient rats do not develop dystrophic lesions histologically, even though other muscles in the same animals, not denervated, do develop this myopathy. The present study was conducted to determine whether or not muscle inactivity alone rather than denervation may account for the resistance to vitamin E deficiency lesions. Electromyographic signs of vitamin E deficiency were studied in rabbits skeletal muscles (1) denervated by peripheral nerve section, (2) with spinal cord section but with intact lower motor neurons, and (3) with tenotomy but with upper and lower motor neuron pathways intact. Spinal cord section or tenotomy did not alter the appearance of electromyographic findings (fibrillation and dystrophic potentials) which occur as dystrophy of vitamin E deficiency develops. In denervated muscles the electromyographic picture of widespread, continuous fibrillation potentials which occurred did not change as the vitamin E deficiency developed. At the same time, contralateral non-denervated muscles in these same animals had positive electromyographic findings of dystrophy.

13

Pharmacology

SOME EFFECTS OF RYANODINE ON GLYCEROL EXTRACTED RABBIT PSOAS FIBERS. Wilford L. Haslett* and Donald J. Jenden. UCLA Med. Center, Los Angeles, Calif.

It has previously been reported that ryanodine fails to interfere with ATP-induced contraction or subsequent EDTA-induced relaxation in glycerol extracted rabbit psoas fibers (Blum et al, J. Pharmacol. 121: 477, (1957)). These findings have been confirmed, but conditions have now been found in which profound contraction occurs in response to ryanodine in a concentration (10^{-5} M) at which a comparable effect is seen in intact muscle. Bundles of psoas fibers were suspended in 250 ml. 50 %w/v glycerol at 0° C with continuous agitation for 24-48 hrs. Strips of these 70-100μ in radius were mounted for isometric recording in 0.1 M KCl, 0.1 M N-cyanoethyl-tris-(hydroxymethyl)-amino-methane buffer at pH 6.2, 4×10^{-3} M $MgCl_2$ and 10^{-3} M mercaptoacetic acid. Addition of ATP (1-5 mM) induced rapid contraction immediately followed by almost complete relaxation. Ryanodine now produced maximal contraction (compared with Ca++) in a concentration of 10^{-5} M or more. A higher concentration of ryanodine is required in the presence of a higher concentration of ATP. Subsequent partial or complete relaxation may be obtained, without removing the ryanodine, with creatine phosphate (10mM), excess ATP, washed microsomes, EDTA (5 mM) and p-hydroxy-mercuribenzoate (2mM). (Supported by a grant from the United States Public Health Service.)

1

169. MUSCLES–Metabolism

Physiology

NEUROMUSCULAR DISORDERS AS ELICITED BY DIETARY CHLORIDE EXCESS OR DEFICIENCY. E. Bajusz* and H. Selye, Institut de Médecine et de Chirurgie expérimentales, Univ. de Montréal, Canada.

An otherwise ineffective oral dose of Na_2HPO_4 (0.25mM/100gm) produces intensive muscular cramps in the rat (generalized continuous tremor and occasional fits of generalized clonic convulsions) when the chloride content of the diet is either increased to 1.62% or decreased to 0.03%, from a basic 0.93%. In rats kept on high chloride diet for 14 days, subcutaneous injection of 9α-fluorocortisol (250-300μg/100gm) produces a qualitatively different type of neuromuscular dysfunction, with only a slight tremor but rather persistent, tonic extensor cramps, predominantly in the hind legs. Sensitization with low chloride diet not only significantly aggravates the calcium deposition in the muscular tissue due to combined dihydrotachysterol (50μg/100gm/day) + Ca-acetate (0.25mM/100gm/day) administration, but in the rats so treated acute paralysis develops. Further studies concerning the role of certain anion-cation interactions in the pathogenesis of neuromuscular diseases, and especially the influence of the variations in the sodium/chloride ratio of the diet will be discussed. (Supported by G. and A. Pfeiffer Found. and Gr. B2037, Ntl. Inst. Neurol. Dis. Blindn., U.S.P.H.)

2

Physiology

METABOLIC -ELECTRICAL INTERRELATIONSHIPS OF FROG MUSCLES. L. G. Abood, K. Koketsu*, and K. Noda*. Dept. of Psychiatry, Univ. of Illinois, Chicago.

With the use of metabolic inhibitors such as dinitrophenol (DNP) it has been possible to investigate the relationship of metabolic to electrical and electroionic effects in frog sartorius muscles. Muscles were soaked for 2 hours in ringers or sodium-free hydrazinium (0.1 M) solutions containing orthophosphate-P^{32} and varying concentrations of DNP (0.01-1.0 mM). While the inhibition of phosphorylation (Δ P) varied from 40-100% at such concentrations of DNP, there was as much as a 4-fold increase in oxidation and glycolysis. Accompanying the inhibition of Δ P was a marked increase of intracellular Na^+ and decrease of K^+. At 1 mM DNP all changes were maximal and the resting potential dropped below 50 mV with complete disappearance of electrical activity. At this stage, however, it was possible to restore electrical excitability by anodal polarization applied through intracellular electrodes. Although a concentration of 0.1 mM DNP in either ringers or hydrazine solution virtually eliminated Δ P, the electrical activity was normal in the former and absent in the latter solution. Such findings suggest the almost complete independence of phosphorylation and excitability.

3

Biochemistry

METABOLIC STUDIES ON RAT MUSCLE FIBERS. R. D. Peterson, C. H. Beatty,* H. H. Dixon* and E. S. West. Dept. of Biochemistry, Univ. of Oregon Med. School, Portland, Oregon.

Myographic and clinical observations indicated that anxiety patients show added fatigue after continued administration of chlorpromazine (CPZ). This apparent energy deficit may be related to inhibition of oxidative phosphorylation by CPZ. The effect of CPZ on metabolism of adductor muscle fibers from depancreatized rats was investigated (Krebs bicarbonate buffered medium plus 150 mg % glucose, pH 7.4). The table summarizes control levels of constituents analyzed and percentage changes with varying concentrations of of CPZ. $< P .025$ to $< .005$ for all changes reported. 0 indicates no significant change.

2 hours incubation	Control	Chlorpromazine		
	mg/100 g as P	1 mg %	5 mg %	10 mg %
		% decrease in 2 hrs incubation		
ADP + ATP	32 ± 1 SE	0	18	71
Phosphocreatine	44 ± 2	0	38	80
Production	mg/g/hr			
Lactate	1,6 ± 0.01	0	0	0
Pyruvate	0.14 ± 0.01	0	50	100
Release		% increase in 2 hrs incubation		
Amino N	0.15 ± 0.01	0	31	110

These data show that CPZ interferes with maintenance of energy

Aided by PHS Grants M-2263, A-1157 and A-213.

4 *Biochemistry*

CRYSTALLIZATION OF HUMAN MUSCLE PHOSPHORYLASE.
Adel A. Yunis* Edmond H. Fischer and Edwin G. Krebs. Dept. of Biochemistry, Univ. of Washington, Seattle, Washington.

The recent discovery of a human myopathy with lack of muscle phosphorylase adds great interest and potential importance to the isolation of this enzyme. Phosphorylase b from human skeletal muscle obtained at autopsy has been extensively purified by ammonium sulfate fractionation and column chromatography on DEAE cellulose. A seventy-fold purification was achieved yielding a fraction having a specific activity of 1100 units/mgm (Cori units) at pH 6.8. The enzyme could not be crystallized under conditions similar to those used in the crystallization of rabbit phosphorylase b, i.e., with Mg^{++} and AMP. When human phosphorylase b was converted to phosphorylase a with purified rabbit phosphorylase kinase, crystallization occurred readily upon dialysis against 0.02M-0.012M Glycerophosphate-cysteine buffer at pH 6.8. Crystalline human phosphorylase a showed one peak on electrophoresis and ultracentrifugation and had a sedimentation constant of 14.4 which was twice that found for human phosphorylase b. It had a specific activity of 1600 units/mgm, a pH optimum between 6.5 and 6.8, and showed 85% of its activity without added AMP. Spectrophotometric analysis showed the presence of 4.3 moles of pyridoxal phosphate per mole of phosphorylase a, assuming a molecular wt. of 500,000, as found for rabbit phosphorylase a.

5 *Biochemistry*

PHOSPHORYLTRANSFER WITH PHOSPHOCREATINE (PC) OR PHOSPHOENOLPYRUVATE (PEP) AND ADENOSINEMONOPHOSPHATE (AMP). J. Molnar * and L. Lorand , Dept. Chemistry, Northwestern University, Evanston, Ill.

From experiments with tissue extracts, the Lohmann reaction (I) (2PC+AMP=2 creatine (C)+ATP) has been interpreted in terms of an ATP-creatine transphosphorylase (A) step (PC+ADP=C+ATP) coupled to one catalyzed by myokinase (B) (ATP+AMP=2ADP). It has been assumed that in purified systems ADP would be an obligatory primer (Chappel and Perry; Oliver). We now find that (I), as judged by the appearance of C and disappearance of PC, proceeds well without the addition of ADP in the presence of A and B even if these are of crystalline purity and are dialyzed exhaustively. PEP and its kinase can replace PC and A in (I). Prior treatment of the reaction components with anyrase does not affect (I); an evidence against the presence of free ADP or ATP. Furthermore, crystalline A catalyzes a slow but definite exchange between C^{14}-creatine and PC ($PC+C^{*}=PC^{*}+C$), the rate of which appears to be related to the conventional enzymatic activity of A. (Supported by a grant-in-aid from the Muscular Dystrophy Associations of America, Inc.)

6 *Pharmacology*

DISTRIBUTION OF ACETYLCHOLINESTERASE (AChE) ACTIVITY IN HUMAN MUSCLE. J. C. Smith*, V. Foldes* and F. F. Foldes. Dept. of Anesthesiology, Mercy Hospital and Section on Anesthesiology, Department of Surgery, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania

Biopsied human muscle was immediately homogenized in 0.25M sucrose and separated into 4 fractions by differential centrifugation. AChE activity, with ACh substrate, was determined in each fraction with a pH-Stat. Of the total AChE activity, the microsomal and mitochondrial fractions contained 33% and 11% respectively; 35% was present in the cellular debris and 21% in the supernatant fluid. If specific AChE activity, with reference to protein, of the homogenate was taken as 1.0, then the specific activity of the cellular debris and supernatant fluid was 0.5 and that of the microsomal and mitochondrial fractions 45.0 and 5.0 respectively. The activity of the cellular debris could not be released by freezing or other disruptive techniques. AChE activity of the supernatant fluid probably represents contamination by blood. (Supported by grant from Health Research and Services Foundation, Pittsburgh)

170. MUSCLES–Myoneural Junction

1 *Physiology*

ELECTRICAL ACTIVITY IN FIBRILLAR-TYPE MUSCLE FIBERS. Frances V. McCann (intr. by R.E. Gosselin.) Physiol. Dept., Dartmouth Med. Sch., Hanover, New Hampshire, and U.of Conn., Storrs,Conn.

Single indirect flight muscle fibers of insects representing 4 orders (Diptera, Hymenoptera, Hemiptera, Coleoptera) were examined with intracellular microelectrodes to evaluate and compare electrical responses. Resting and action potentials elicited by electrical stimulation of nerve and the effects of several chemicals (CO_2, ether, CCl_4) were measured and compared. Insects of the orders Diptera and Hymenoptera gave a uniformly rising, overshooting action potential. Ether and CCl_4 affected the CNS and the myo-neural transmission process, while CO_2 produced a rapid depolarization of the muscle fiber membrane. The Hemiptera characteristically showed a slower compound action potential, a transmission block by ether and CCl_4 but no depolarization with CO_2. The Coleoptera frequently gave junction-type potentials showing summation and facilitation, and an insensitivity to the chemical agents. It is concluded that fibrillar muscle as it is found in the Coleoptera invests unique features not found in the other orders studied.

2 *Physiology*

BLOCKING ACTION OF THIAMINE ON NEUROMUSCULAR TRANSMISSION AND MEMBRANE CONDUCTION. W. L. Nastuk and N. Kahn.* Dept. of Physiology, Columbia University, New York.

Thiamine (thi) applied to isolated, neurally stimulated, frog sartorius-sciatic nerve preparations, reduced twitch tension without potentiation. The reduction was: just perceptible at thi 1 mM, 50% at thi 2 mM, and 100% at thi 3 mM. Endplate sites in isolated frog sartorius muscles were microperfused to detn. the threshold conc. of acetylcholine (ach) required to cause twitching (4 mgm/L). For muscles in Ringer sol'n plus thi 2 mM and 3 mM, the respective ach thresholds were 6.5 and 19.7 mgm/L. Transmembrane potentials were recorded at endplates of fibers from muscles equilibrated in thi-Ringer sol'n and neurally stimulated. For 13 endplates in 1 muscle at 1 mM thi, the resting potential (RP) was 99.9 mV, the action potential (AP) was 133.9 mV, and 3 junctions were blocked. For 40 endplates in 4 muscles at 2 mM thi, RP = 94.7 mV, and 37 junctions were blocked. Endplate potentials recorded at 24 blocked junctions were 11.1 mV in amplitude, rose to peak in 1.4 msec, and decayed 50% in 4.4 msec. Fibers (13) of 2 muscles equilibrated in thi 4 mM showed RP = 89.3 mV at non-endplate sites, and their excitability diminished with time. Those AP's elicited showed reduction in amplitude and in rates of rise and fall. Supported by NSF Grant #8812.

3 *Pharmacology*

THE NEUROMUSCULAR ACTIVITY OF TOXIFERINE IN UNANESTHETIZED MAN. B. Wolfson*, F. F. Foldes, H. M. Brunn, Jr.* and O. Herrera*. Dept. of Anesthesiology, Mercy Hospital and Section on Anesthesiology, Department of Surgery, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania.

Fifteen microgm/kg toxiferine, a synthetic, non-depolarizing neuromuscular blocking agent, was injected intravenously in 90 seconds to 10 healthy, adult male and female volunteers. Grip strength was completely abolished in 8 of the 10 subjects and vital capacity decreased to a mean of 54.4 ± 5.0 per cent of control within 6 minutes. Vital capacity returned to 75.8 ± 4.0 per cent of control in 11.5 minutes, grip strength, however, was still only 63.7 ± 5.8 per cent at 39.6 minutes. Exercise of the partially curarized muscle increased the intensity and duration of the neuromuscular block. The neuromuscular effects of 15 microgm/kg toxiferine could be antagonized by 0.02 mg/kg neostigmine. Toxiferine had no appreciable effect on circulation and after intravenous injection or intradermal skin test, showed no evidence of histamine release.

4 *Pharmacology*

DEPRESSION OF MAMMALIAN NEUROMUSCULAR TRANSMISSION. A. S. Luperman (intr. by W. F. Riker, Jr.) Dept. of Pharmacology Cornell Univ. Med. College, New York City.

Preliminary study signified that agents primarily characterized by their facilitatory action may also produce neuromuscular depression by a variety of mechanisms. Diminution or enhancement of the neurally elicited twitch response of cat gastrocnemius served as a signal for neuromuscular depression and facilitation. The biphasic action of methonium facilitatory agents has been well established and the depressant phase is viewed as excessive endplate depolarization. However, ethonium facilitatory drugs were also observed to elicit twitch depression; this depression became particularly obvious with increasing doses and stimulus durations that induced repetitive nerve firing. Furthermore, agents like 3-OH phenyltrimethyl and phenyldiethylmethylammonium, in doses subthreshold for facilitation, produced a limited diminution of twitch height. This exceeded depression caused by equivalent doses of the depolarizing ion, phenyltrimethylammonium. Obviously, neuromuscular depression by quaternary ammonium ions is not always adequately explained within the context of an endplate depolarizing or stabilizing action. The evidence suggests that a depressant effect is a concomitant property of all facilitatory agents and is exerted at a presynaptic locus.

5 *Pharmacology*

EFFECT OF AGE ON RESPONSE TO SUCCINYLCHOLINE IN MAN. Arthur S. Keats and Jane Telford*. Dept. Anesthesiology, Baylor Univ. College of Medicine, Houston, Tex.

In 776 Patients (ages 2 weeks to 45 years) undergoing thoracic surgical procedures, the amounts of succinylcholine required to maintain apnea were measured. An inverse relationship was noted between age in years and mcg/kg/min. of succinylcholine to maintain apnea. To produce equal effect, 4 times as much succinylcholine was required in the infant (0-3 month) group as in the adult (20+ years) group. A smooth non-linear curve expressed this relationship. The apnea time following single intravenous doses of succinylcholine (1.2 mg/kg) was measured in 104 patients (ages 3 weeks to 43 years). A similar relationship was obtained. Apnea in the adult group was 4 times as long as in the infant group. Assuming the same number of myoneural junctions in infants as in adults, these data support the hypothesis that the dose of succinylcholine required for complete paralysis is related primarily to the number of myoneural junctions.

6 *Pharmacology*

EFFECTS OF SUCCINYLCHOLINE, C_{10} AND AN OXAMIDE, WIN 8078, AT THE NEUROMYAL JUNCTION. K. C. Kim*, Alexander G. Karczmar and K. Kokotsu*. Dept. of Pharmacology and Therapeutics, Stritch School of Med. of Loyola University and Dept. of Psychiatry, University of Illinois College of Med., Chicago, Illinois.

An oxamide, WIN 8078, was shown to antagonise d-tubocurarine and to convert succinylcholine (SCh) and C_{10} blockade into neuromyal facilitation (SCh and C_{10} "reversal", Karczmar, J. Pharmacol. and Exp. Therap., 119: 301, 1957). This does not agree with the concept that depolarisation constitutes the mechanism of C_{10} and SCh blockade, nor with the possibility that WIN 8078 acts as an anticholinesterase at the neuromyal junction. It was shown at present that C_{10} and SCh first depolarise and then desensitise frog sartorius against acetylcholine (ACh). Mechanical blockade coincides with the second rather than first phase of action of C_{10} and SCh (cf. Thesleff, Acta Physiol. Scand., 34: 218, 1955). Depending on concentration, WIN 8078 sensitises for or competes with, ACh depolarisation; it also increases frog sartorius endplate potential but does not prolong it contrary to eserine or prostigmine. The relationship of these multiple aspects of action of WIN 8078, SCh and C_{10} is discussed with reference to WIN 8078 "reversal." (Supported in part by PHS Grant B-1368.)

7 *Pharmacology*

STUDIES ON THE PHARMACODYNAMICS OF OXIMES IN ORGANOPHOSPHORUS ANTICHOLINESTERASE POISONING. Edmund Bay (intr. by R. V. Brown) Army Chemical Center, Md.

In the treatment of severe organophosphorus anticholinesterase poisoning 2-pyridine aldoxime methiodide (2-PAM) and 1-1'-trimethylene bis (4-formylpyridinium bromide) dioxime (TMB-4) appear to be the most promising adjuncts to atropine. Since at therapeutic dose levels in control animals these oximes alter the function of the neuromuscular junction, pharmacological actions other than reactivation of the inhibited enzyme seem to be involved in successful treatment. To elucidate further the pharmacodynamics of these compounds at the myoneural junction, experiments were conducted in several species by recording the isometric tension of the Achilles' tendon muscle. The results suggest that 2-PAM, under experimental conditions, has a "dual" or "biphasic" action which appears as a characteristic short depolarizing and longer lasting "curare-mimetic" effect. The mechanism of action of TMB-4 is similar to that of 2-PAM. It differs in the lack of the initial depolarizing effect. It is concluded that in the rapid reversal of neuromuscular block caused by organophosphorus anticholinesterases, a direct antagonism to acetylcholine at receptor sites is also involved besides the previously reported reactivation of inhibited cholinesterase enzyme.

171. MUSCLES—Physical Properties

Biochemistry

SPECTROPHOTOMETRIC TITRATION STUDIES OF MYOSIN AND THE MEROMYOSINS. A. Stracher (intr. by R. R. Becker.) State University of New York Downstate Medical Center, Brooklyn, N. Y.

Spectrophotometric titration studies of myosin and L- and H-meromyosin in 0.05 M piperidine buffer have shown that in myosin, approximately 35% of the tyrosine residues are anomalous, in that they cannot be titrated until a very high pH (above 12) is reached. The remaining tyrosine residues titrate normally (pK 10.5.) Back titration from pH 12.8 to pH 7.3 indicates that the effect is irreversible since all of the tyrosine residues now titrate normally. With L-meromyosin, 90% of the tyrosine residues titrate above pH 12 and the effect is irreversible, whereas with H-meromyosin the majority of the tyrosines titrate normally and reversibly. These results would indicate that the breakdown of myosin into sub-units at high pH is caused by rupture of hydrogen bonds between tyrosine and some acceptor group in the molecule. It is concluded that this type of bonding may be a major factor in the stabilization of the tertiary structure of myosin and L-meromyosin.

173. NEUROANATOMY—Surgical Procedures

Physiology

BLOOD PRESSURE CHANGES IN ARTERIES SUPPLYING THE BRAIN. Williamina A. Himwich, Giovanni Ayala*, JoAnn Petersen* and Juanita Graves*. State Research Hospital, Galesburg, Illinois.

The changes in the hemodynamics of the dog brain after ligation of the carotid and/or the vertebral arteries, permanent clipping of arteries within the brain and the anastomosis of vertebral and carotid circulations have been studied with catheters implanted in one or both lingual arteries. The electroencephalogram and the accompanying changes in systemic arterial and venous blood pressures were also followed. The clamping of a common carotid artery results in an immediate fall in pressure in the ipsilateral lingual followed by a slow movement of the pressure toward recovery. Subsequent clamping of the external carotid artery anterior to the lingual artery and of the internal carotid artery on the same side give indirect measures of the contribution of the pressure in these vessels to the pressure in the Circle of Willis. Simultaneous clamping of both the vertebral artery and the common carotid artery on the same side reduces the pressure in the ipsilateral lingual with little tendency to recovery. A better return of pressure occurs after the vertebral and carotid arteries are anastomosed. The physiological implications of these changes in hemodynamics will be discussed.

XUM

174. NEUROPHARMACOLOGY

Physiology

CEREBRAL SYNAPTIC INHIBITION BY HISTAMINE. Thomas M. Gilfoil,* E. Ross Hart* and Amedeo S. Marrazzi. Vet. Admin. Research Labs. in Neuropsychiatry, Pgh. 6, Pa.

The ubiquity and ready availability of histamine in the body make the question of its effect on synaptic transmission highly pertinent. We have therefore studied the effect on transcallosally evoked potentials in the optic cortex of the nembutalized cat of close arterially (intracarotid) injected histamine. This makes possible comparison to our previous data, so obtained, showing the inhibitory action of adrenaline, noradrenaline and serotonin. As with these the reduction in potentials, evoked by constant submaximal electrical stimuli, is evidence that histamine is a potential cerebral synaptic inhibitor of the order of potency of serotonin or better. The possible role of the marked depressor action of the 1-4 μg/Kg. used has been minimized by demonstrating that acetylcholine producing similar B.P. depression does not inhibit (but rather excites). The specificity of the synaptic inhibition is shown by its prevention with an antihistamine(tripelennamine). The latter, furthermore, can on occasion show a differential protection of the synapse, permitting fall of B.P. without synaptic inhibition. Attempts are in progress to determine the effect of histamine liberated in situ. A new cerebral synaptic neurohumoral inhibitor is suggested.

Physiology

EXPLANATION OF ADRENERGIC POTENTIATION AND BLOCKADE BY CHOLINERGIC MECHANISMS. Melville W. Osborne, Irene L. Woessner and Katherine Naeff (intr. by W.W. Swingle). Geigy Research Labs., Ardsley, New York.

Evidence in anesthetized cats and dogs indicate that agents involving adrenergic mechanisms (cocaine, imipramine and methylphenidate) possess a common pharmacodynamic identity by acting as cholinesterase inhibitors. Interaction of these compounds with catecholamines and other sympathicomimetic agents are regarded as that between two cholinesterase inhibitors. Depending on the titers in which they are presented to each other, they enhance or diminish autonomic effector responses: Low doses of imipramine potentiate the vasopressor response and nictitating membrane contraction to catecholamines; high doses depress these responses. The effect of peripheral vagal stimulation and acetylcholine induced vasodepression is also enhanced after low doses of cocaine, imipramine and methylphenidate. During reserpine evoked release of catecholamines administration of imipramine produces a strong "ganglionic drive" during which chlorisondamine fails to induce blockade. It is proposed that potentiation or depression of adrenergic phenomena is governed by the type and titer of the cholinesterase inhibitor introduced.

Physiology

LSD ALTERATION OF OPTIC POTENTIALS (CAT LATERAL GENICULATE); BLOCK BY SCHIZOPHRENIC SERUM. Stephen S. Fox (intr. by R. W. Gerard) University of Michigan

Small amounts of LSD injected I. V. into cats with electrodes in the lateral geniculate, 25 acute (curarized and respirated) and eight chronically implanted, significantly alters the evoked potential from light flashes at 4/sec. The change is seen as a 25-35% increase in peak-to-peak amplitude, from 150 v average to 200 v. The wave form changes under LSD with an increase in the positive component and either no change or slight diminution in the negative component. Spontaneous activity following evoked activity also increases. Some distortion of the wave is also noticeable. In 13 out of 15 acute cases and in seven out of eight chronic cases, 5 cc. of serum from schizophrenic subjects essentially blocked the LSD effect; one non-schizophrenic serum of 15 showed this effect. All subjects were males of like age diagnosed by three concurring psychiatrists and were under like conditions-diet, drug withdrawal, activity, ward environment, etc. - for 4-12 weeks before testing. (Supported by U.S.P.H.S.)

Physiology

POTENTIATION OF ADRENERGIC AND CHOLINERGIC MECHANISMS WITH SMALL DOSES OF IMIPRAMINE. Ernest B. Sigg, Melville Osborne* and Elliot Bindler* Geigy Research Labs., Ardsley, New York.

It was reported (J.Can.Psychiat.4:575, 1959) that imipramine potentiates the effect of norepinephrine on blood pressure and nictitating membrane. Experiments in anesthetized cats and dogs show that small doses (100 to 320 γ/kg i.v.) also enhance the vasodepressor response to acetylcholine and effect of peripheral vagal stimulation. Potentiation of the response to DMPP is observed in parasympathetic (gut and bladder) and sympathetic (nictitating membrane) effector organs. During reserpine induced release of catecholamines imipramine causes marked hypertension. The carotid occlusion reflex is slightly augmented. Cortical and hypothalamic brain wave activity exhibits fast frequency - low amplitude pattern. Spinal reflexes are only inconsistently altered. In reserpinized spinal cats imipramine causes intense increase in muscle tone. The dose levels used correspond well with the blood levels of imipramine in patients under the treatment.

Physiology

INHIBITION OF SOUND-INDUCED RUNNING, CLONUS, AND TONUS BY VARIOUS CENTRAL NERVOUS SYSTEM DEPRESSANTS. E. A. Conrad (intr. by L. Grumbach.) Pharmacology Sect., Sterling-Winthrop Res. Inst., Rensselaer, N.Y.

Mice susceptible to audiogenic stimulation often show running, clonus and tonus as components of their reaction syndrome. The observed clonus and tonus suggested the possible usefulness of such animals for evaluation of potential anticonvulsant agents, thus supplementing or replacing the widely used maximal electroshock(MES) and psychomotor(PsM) tests. Thirteen drugs used clinically as anticonvulsants, tranquilizers or central relaxants were tested for their ability to counteract audiogenic, MES and PsM seizures. Several points of interest were noted. The running phase of the audiogenic seizure is more difficult to suppress than clonus or tonus whereas tonus is inhibited most readily. Rank order correlation coefficients were determined for protection against MES vs. audiogenic tonus, and inhibition of PsM vs. audiogenic clonus; they were 0.903 and 0.782 respectively ($P = <0.05$). Reserpine failed to protect against audiogenic seizures. High doses of chlorpromazine inhibited all components of the sound-induced reaction.

Pharmacology

AMINO ACIDS AND SPINAL NEURONES. D.R. Curtis, J.W. Phillis and J.C. Watkins (intr. by C. McC. Brooks) Australian National University, Canberra.

A variety of amino acids have been applied ionophoretically to spinal interneurones, motoneurones and Renshaw cells of the cat. In most cases spike potentials have been recorded extracellularly but the action of some acids has been determined upon intracellularly recorded potentials of motoneurones. Certain neutral amino acids related to gamma aminobutyric acid depress spinal neurones without changing the membrane potential. It is considered that the activity of these substances is unrelated to inhibitory synaptic action but is associated with an increase in the membrane conductance. Acidic amino acids related to glutamic acid excite spinal neurones by producing a membrane depolarization. This action is probably unrelated to excitatory synaptic action.

7 *Pharmacology*

ANTICONVULSANT ACTION OF METHAMINODIAZEPOXIDE HCl. E. F. Keith and G. A. Ardoline (intr. by W. B. Schallek) Dept. of Pharmacology, Hoffmann-La Roche, Inc., Nutley 10, N.J.

A new compound, Librium (T.M.) hydrochloride, with the generic name methaminodiazepoxide HCl, and chemically 7-Chloro-2-methylamino-5-phenyl-3H-1,4-benzodiazepine 4-oxide hydrochloride has shown several interesting pharmacologic actions. Among these is a marked anticonvulsant activity. In three experimental models of the convulsive states in mice this material is active by all routes of administration studied so far. When administered by the oral route it is more active against pentylenetetrazol induced convulsions than diphenylhydantoin sodium, which was inactive at 800 mgm/kgm, and phenobarbital sodium. Against supramaximal electroshock seizures it was slightly less active than both. In the minimal electroshock model it was more active than diphenylhydantoin and less active than phenobarbital. Duration of action is in excess of five hours in mice. Details of difference in action of methaminodiazepoxide HCl and other substances will be discussed.

8 *Pharmacology*

THE PHARMACOLOGY OF A NEW OXAZOLIDINONE WITH ANTICONVULSANT ANALGETIC AND MUSCLE RELAXANT PROPERTIES. M.N.Carroll, Jr. W.R.Luten* and R.W.Southward*. A.H.Robins Biol.Res.Labs., and Dept. of Pharmacology, Med.Coll.of Virginia, Richmond.

In the search for a centrally acting skeletal muscle relaxant of high potency, long duration and minimal toxicity, 5-(3,5-dimethylphenoxymethyl)-2-oxazolidinone(AHR-438) exhibited superior activity against electrically and chemically(strychnine) induced convulsions in rats. The sedative, hypnotic and ataractic properties of this compound appeared minimal as determined by effects on prehensile-traction reflexes (mice), mescaline induced scratch antagonism (mice), hexobarbital narcosis potentiation (mice) and electroencephalographic studies in unanesthetized chronically implanted cats. AHR-438 demonstrates superior anti-pressure analgesia and antagonized alloxan induced "writhing" in rats. In lightly anesthetized-intact cats, the compound blocked the polysynaptic linguo-mandibular and flexor reflexes without effect on the monosynaptic patella reflex or the muscle twitch response at doses which didn't alter normal posture and gait in unanesthetized animals. Moreover, this compound altered the effects of contralateral sciatic stimulation (crossed extension) as well as mesencephalic reticular modifications of the patella reflex. AHR-438 appears to be an effective analgesic and skeletal muscle relaxant with minimal sedative properties.

9 *Pharmacology*

PHARMACOLOGY OF AMPHENIDONE (DORNWAL) A NEW PSYCHOPHARMACOLOGICAL AGENT. J.F. Reinhard, O.J. Plekss*, and J.V. Scudi. Maltbie Labs. Division, Wallace & Tiernan Inc., Belleville,NJ.

The clinical effectiveness of 1-m-aminophenyl-pyridone-2-(amphenidone) prompted this report of its pharmacological properties. Sedative-hypnotic activity could not be demonstrated except at lethal doses, and then only in rodents. Stimulant effects of intracerebrally injected LSD were completely blocked by low doses of amphenidone. Electrically (MES) and chemically (Metrazol, Strychnine) induced seizures were prevented. Anticonvulsant potency, especially vs. Metrazol, equalled that of reference anti-epileptics. Analgesic potency (Eddy technic) was 1/3 that of codeine. Flexor (polysynaptic) reflexes were depressed in anesthetized (chloralose) and spinal (C-2) cats. Patellar (monosynaptic) reflexes were not significantly affected. Cord-depressant activity was comparable to, but longer lasting than mephenesin. Blood pressure and respiration in the dog were affected only at lethal doses. Intravenous doses up to 100 mg/kg did not alter blood pressure responses of acetylcholine, epinephrine, norepinephrine or serotonin, or the ECG (lead 2). Normal functioning of the isolated heart was not altered by the addition of 1000 µg to the perfusion fluid. It is concluded that amphenidone has selective activity on the CNS. These findings parallel those reported in clinical trials.

10 *Pharmacology*

EFFECTS OF RESERPINE AND IPRONIAZID ON HYPOTHALAMIC NEUROSECRETION- Bruno Minz.Lab.Pathol.Gén.Fac.Med.Univ.of Paris,France.

It has been previously shown through pharmacodynamic analysis and transections at different central levels that the local application of a concentrated epinephrine solution on the fronto-parietal zone of the rabbit cerebral cortex initiates the liberation of a vasopressor substance from the anterior and that of an oxytocic agent from the posterior part of the hypothalamus. It seemed of interest to investigate whether and to what extent these diencephalic reactions might be modified under the effect of psychotropic drugs such as reserpine and iproniazid. The experiments were performed with adult female rabbits, ovariectomized and premedicated with 10 microgr of oestrine daily for 10 days, blood pressure and the spontaneous activity of the uterus in situ were recorded simultaneously. It has thus been fount that systemic pretreatment with reserpine enhances the cortically induced pressor effect of epinephrine, but inhibits the oxytocic response to the same hormonal stimulus. Iproniazid, on the contrary, inhibits the hypertensive response without altering the oxytocic process. Reserpine and iproniazid are susceptible of blocking specifically and in an antagonistic way biochemical reactions at the level of two different hypothalamic areas.

11 *Pharmacology*

ASPECTS OF DEPRESSANT DRUG SELECTIVITY FOR SPINAL MONOSYNAPTIC AND POLYSYNAPTIC RESPONSES. Don W. Esplin. Dept. of Pharmacology, Univ. of Utah, Salt Lake City, Utah.

Striking differences in selectivity are observed with prototype depressant drugs: mephenesin selectively reduces polysynaptic activity, carbon dioxide preferentially decreases the monosynaptic spike, whereas general depressants, such as pentobarbital, affect both types of discharge. There is little evidence that such agents have specific affinity for discrete neural structures, or that the drugs interfere with neurohumors in the pathways concerned. Accordingly, an attempt has been made to explain the observed selectivity without assuming such actions. From a variety of spinal cord experiments, in which both single- and multiple-unit discharges have been studied, it appears that drug depression has two conspicuous facets: depression of excitation per se and depression of synaptic recovery. Such aspects of depression, considered in light of the functional organization of the spinal cord, provide the basis for suggestions regarding mechanisms underlying the selectivities of the prototypes mentioned. Specifically, it is proposed that selective depression of polysynaptic activity may be associated with marked depression of synaptic recovery, and consequent reduction of repetitive interneuron discharges. (Supported by Senior Research Fellowship SF-145 and grant B-872, N.I.H.)

12 *Pharmacology*

ANALYSIS OF THE SPINAL EXCITATORY ACTION OF PENTYLENETETRAZOL. Jorge Lewin* and Don W. Esplin. Dept. of Pharmacology, Univ. of Utah, Salt Lake City, Utah.

Pentylenetetrazol (Metrazol) was studied in unanesthetized spinal cats by conventional electrical recording technics. In doses of 20 to 50 mg/kg i.v., the drug increased polysynaptic activity and decreased monosynaptic (2N) responses. Metrazol modified synaptic recovery in the 2N pathway in a diphasic manner: recovery was increased with intervals between conditioning and test stimuli less than 200 msec. and was decreased with longer intervals. The increase in synaptic recovery cannot be attributed to an action of Metrazol on the motoneuron because the drug did not modify recovery after an antidromic conditioning stimulus. Therefore it appears that Metrazol has a presynaptic action. In addition to this effect, the drug strikingly increased recurrent inhibition through stimulation of Renshaw cells. This effect, together with the reduction in 2N discharge, indicates that Metrazol stimulates inhibitory as well as excitatory systems in the spinal cord. The significance of this finding with respect to the locus of action of Metrazol will be discussed. Significantly, it has been found that trimethadione antagonizes all the actions of Metrazol described. (Supported by Rockefeller Foundation Fellowship, and by Senior Research Fellowship SF-145 and grant B-872, N.I.H.)

XUM

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Pharmacology

BIOCHEMICAL ASPECTS OF "LSD-PSYCHOSIS." D. V. Siva Sankar (intr. by F. Cotui.) Creedmoor State Hospital, Jamaica 27, New York, N. Y.

We have carried out comparative investigations on the enzymatic and metabolic effects of the hallucinogenic drug, lysergic acid diethylamide (LSD) and its non-hallucinogenic bromo analog (BOL). The enzymatic studies were made using rat cerebral and cerebellar homogenates in a buffered Krebs Ringer bicarbonate medium. LSD enhanced the oxidation of glucose, succinate and citrate by the cerebral preparations, while it either depressed it or did not have marked effect on the cerebellar enzymatic activity. The effects of reserpine and serotonin were similar to those of LSD while the action of BOL was different. Further details and the effect on amine oxidase and amino acid decarboxylase activity will be presented. The metabolic effects of LSD and BOL were investigated using intact rats in metabolism cages. The most important effect of LSD (not shown by BOL) was the decrease in urinary urea and keto acid excretion. This may possibly involve oxidative metabolism as related to liver function. These studies show somatic involvement in an experimentally produced "psychosis." Studies have been made on the urinary excretion of phosphate, phenolics, amines, metabolites of tryptophan-3-C^{14}, etc. The results of these studies will be presented and discussed.

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Pharmacology

ANTICONVULSANT PROPERTIES AND METABOLIC ASPECTS OF N-ALKYL SUBSTITUTED BENZILIC AMIDES. J. R. Albert* and J. H. Weikel, Jr., Dept. of Pharmacol., Mead Johnson & Co., Evansville, Ind.

The anticonvulsant activities of N-alkyl substituted benzilic amides (synthesized by Dr. F. A. Grunwald) were determined in mice by the maximal electroshock (M.E.S.) and subcutaneous metrazol tests. This series was most active against M.E.S. and had potent anti-metrazol activity as well. Benzilic amide itself was potent in both tests, but the dose producing neurological deficit was near the protective dose. N-alkyl substitutions of methyl, ethyl and isopropyl groups decreased M.E.S. potency in that order. Cyclohexyl, tert. butyl, sec. butyl and propyl derivatives were inactive. N-ethyl benzilic amide (NEBA) had optimal activity for both M.E.S. and metrazol tests. NEBA had mixed depressant and stimulant actions at high levels, whereas benzilic amide had a hypnotic action at high but non-lethal levels. An analytic test was developed to estimate total benzilic amides in biological fluids and tissues. A metabolite of NEBA, identified as benzilic amide by mixed melting point, infrared absorption spectra, and counter current distribution, was found in the urine. Mice given repeated daily administration of NEBA became tolerant to minimal electroshock seizure. The development of tolerance paralleled a decrease in benzilic amide brain content. Mice given repeated daily doses of benzilic amide did not show tolerance.

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Pharmacology

SELF-SELECTED BRAIN STIMULATION REWARD THRESHOLDS MODIFIED BY DRUGS. Larry Stein (intr. by J. Seifter.) Wyeth Institute for Medical Research, Philadelphia 1, Penna.

In self-stimulation experiments, animals with permanently implanted electrodes work to stimulate certain brain sites electrically just as animals will work to obtain conventional rewards like food. These self-stimulation procedures have been extended by allowing the animals to regulate the intensity as well as the rate of the stimulation, and arranging conditions so that the animal continuously self-selects his threshold for rewarding brain stimulation. After a few weeks of training, the performance is sufficiently stable to provide a dependable and precise baseline for the evaluation of drugs. With electrodes in the posterior hypothalamus and midbrain tegmentum of the rat, intraperitoneal injections of d- and dl-amphetamine (0.5-1 mg/kg), phenylethylamine (30 mg/kg), and methylenedioxyamphetamine (1.5-3 mg/kg) lowered the reward threshold. In the same sites, chlorpromazine (1.5 mg/kg), reserpine (0.6-1 mg/kg), serotonin (4 mg/kg, SC), and LSD-25 (0.01 mg/kg) increased the reward threshold or knocked out self-stimulation behavior altogether. Sodium pentobarbital (15 mg/kg) also stopped self-stimulation at the peak of its action but, in contrast to the other drugs, lowered the threshold before and after the peak effect.

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Pharmacology

INTERACTION OF LYSERGIC ACID DIETHYLAMIDE (LSD) WITH POLYPEPTIDES WHICH STIMULATE SMOOTH MUSCLE. Carroll M. Smith* and Edward J. Walaszek, Dept. of Pharmacology, Univ. of Kansas Medical School, Kansas City, Kansas.

It has been reported (Krivoy, B. J. Pharm. 12: 361, 1957), that LSD in conc. of 1 mcg/ml and less potentiated the stimulating effect of the neurohumoral polypeptide substance P on the isolated guinea pig ileum. Under similar conditions histamine was not potentiated. It was speculated that this interaction may bear some relationship to the hallucinogenic action of LSD. We have confirmed the potentiation of substance P by LSD and have further shown a similar potentiation of bradykinin and substance A, two smooth muscle stimulating polypeptides which are devoid of central nervous system effects. LSD was added to the organ bath 2 minutes before the addition of the agonist. Potentiation was most clearly seen when the conc. of LSD was 0.03 to 0.1 mcg/ml. LSD in conc. higher than 0.1 mcg/ml consistently gave a marked stimulating effect of its own. All potentiations were easily reversed by washing the ileum with fresh Tyrode's solution. Other known hallucinogens were unable to potentiate the stimulating effect of substance P. It is concluded that this potentiation by LSD probably bears no relationship to its hallucinogenic properties and the mechanism of potentiation is probably due to the inhibitory effect of LSD on enzymes capable of inactivating polypeptides.

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Pharmacology

EFFECTS OF PUFFER POISON ON NEUROMUSCULAR TRANSMISSION. J. H. Fleisher, P. J. Killos and C. S. Harrison (intr. by J. H. Wills.) U.S. Army Chemical Warfare Laboratories, Army Chemical Center, Md.

Injection of 50 microgms. of puffer poison (PP) in saline into the anterior chamber of the rabbit eye in vivo produced a prolonged mydriasis. Degeneration of post ganglionic adrenergic fibers to the eye prior to injection had no effect on the production of mydriasis with PP, suggesting no involvement of adrenergic pathways. Carbamylcholine (0.1 mg/kg.) injected subcutaneously into a rabbit showing mydriasis following intraocular injection of PP produced marked pupillary constriction indicating PP to have little or no effect on postsynaptic cholinergic receptors. Incubation of the isolated rat phrenic nerve-diaphragm preparation in solutions containing as little as 0.06 microgms. PP per ml. produces neuromuscular block accompanied by marked inhibition of acetylcholine (ACh) release. This concentration of PP has little or no effect on ACh formation by rat brain slices. It is concluded that the primary effect of PP on neuromuscular transmission is an inhibition of ACh release.

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Pharmacology

EFFECTS OF THALIDOMIDE ON CENTRAL NERVOUS SYSTEM DRUGS. W. L. Kuhn* and E. F. Van Maanen, Dept. of Pharmacology, Scientific Division, The Wm. S. Merrell Co., Cincinnati, Ohio.

Clinical studies with thalidomide (α-[N-phthalimido]glutarimide) have shown that 100 mg given orally induces sleep in man. Experiments in animals revealed that, unlike the usual sedative and hypnotic drugs, thalidomide does not produce loss of righting reflexes. Symptoms of mild central nervous system depression were observed in mice and dogs with 30 mg/kg intraperitoneally, but loss of righting reflexes did not occur with doses of 4000 mg/kg in mice or 100 mg/kg in dogs. The most prominent effects of thalidomide occurred in combination with other centrally acting drugs. In mice 30-100 mg/kg prolonged sleeping time and reduced the HD_{50} (Hypnotic Dose-50) of hexobarbital and barbital, suggesting that thalidomide does not potentiate barbiturates by blocking metabolic pathways in the liver. Thalidomide had no anticonvulsant activity as judged by the maximal electroshock procedure; however, 100 mg/kg reduced the Anticonvulsant Dose-50's of diphenylhydantoin and phenobarbital in mice by ratios of 1.95 and 2.2, respectively. This dose had no effect on the LD_{50} of diphenylhydantoin and it increased the LD_{50} and HD_{50} of phenobarbital by a factor of only 1.2. Thalidomide did not block the conditioned avoidance response in rats; however, it potentiated this activity and the central nervous system depressant action of chlorpromazine.

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Pharmacology

THE EFFECTS OF MONOAMINE OXIDASE SUBSTRATES AND INHIBITORS ON EVOKED POTENTIALS. Alvin M. Revzin* and Erminio Costa. State Research Hospital, Galesburg, Illinois.

Potentials evoked in hippocampus by stimulation of amygdala were studied in unanesthetized cats; either modified "cerveau isolé" or otherwise intact cats with chronically implanted electrodes were used: Tranylcypromine (SKF-385), 2 mg/kg i.v., caused a small increase in the threshold of the potential. dl-5-HTP also caused a slight depression of the response in doses of 100-200 mg/kg, while l-DOPA or Dopamine were ineffective. Premedication with SKF-385, 2 mg/kg i.v., 90-120 minutes before administration of the other compounds markedly potentiated their effects. dl-5-HTP, 10 mg/kg i.v., was then able to cause a marked drop in rhinencephalic evoked potentials and the previously inactive l-DOPA, 10 mg/kg i.v., also caused a depression of the potentials. The time course of the electrical effects paralleled the behavioral changes seen in the chronic preparations. This potentiation was much more marked in the chronic animals, which suggests that the drug effects on the rhinencephalic electrical phenomena were mediated in part through structures at or below the level of our mid-brain transection. Work bearing on this hypothesis will be reported.

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Pharmacology

BIOCHEMICAL AND EEG CHANGES CAUSED BY (+) ∝ METHYLTRYPTAMINE (IT 403). W. G. Van Meter*, G. F. Ayala*, E. Costa and H. E. Himwich, State Research Hospital, Galesburg, Illinois.

In vitro inhibition of M.O.A. and 5 HTP decarboxylase by IT 403 has been reported by Grieg, et al. (J. Pharmacol. 127: 110, 1959). Brain 5 HT of rats given IT 403 is increased for 3 hrs. after 2 to 5 mg/kg, i.p., 2 hrs. after 20 mg/kg brain 5 HTP decarboxylase is also reduced. Rats and other animals given 10 to 20 mg/kg of IT 403 exhibit behavioral abnormalities and/or locomotor excitation. In rabbits, 2 mg/kg of IT 403, i.v. induces EEG desynchronization enduring longer than 2 hrs. After the basilar artery has been ligated desynchronization is elicited by intravertebral but not by intracarotid injections of equal doses of IT 403. The EEG pattern of "cerveau isolé" preparation is not altered by IT 403 given i.v.. However, d-l methyltryptamine (IT 290) is less effective than IT 403 in causing biochemical changes and EEG desynchronization. This is probably the result of an action taking place in the caudad portion of the brain stem reticular formation. Correlation between biochemical and EEG effects will be discussed with regard to IT 403, M.O.A.I. and amphetamine.

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Pharmacology

THE INHIBITION OF GLYCOLYSIS IN BRAIN BY QUINONES AND DIHYDRIC PHENOLS. GERALD COHEN AND PAUL HOCHSTEIN (INTR. BY M. J. FRUMIN). COLUMBIA UNIV., NEW YORK, N.Y.

THE PRODUCTION OF LACTIC ACID FROM GLUCOSE IN CELL-FREE, CO-FACTOR FORTIFIED HOMOGENATES OF MOUSE BRAIN WAS FOUND TO BE INHIBITED BY A VARIETY OF NATURALLY OCCURRING AND SYNTHETIC QUINONES (E.G., MENADIONE, ADRENOCHROME, P-QUINONE) AND ORTHO AND PARA DIHYDRIC PHENOLS (E.G., EPINEPHRINE, DOPAMINE, 2,5-DOPA, HYDROQUINONE). INHIBITION WAS OBSERVED AT CONCENTRATIONS OF DRUG AS LOW AS 10^{-5}M. IN COMPARISON, NO INHIBITORY ACTIVITY WAS OBSERVED FOR META DIHYDRIC PHENOLS (E.G., RESORCINOL) OR MONOHYDRIC PHENOLS (E.G., PHENYLEPHRINE, METANEPHRINE). FROM THE STRUCTURE-ACTIVITY RELATIONSHIPS AN OXIDATIVE MECHANISM WAS INFERRED. IN AGREEMENT WITH THIS CONCLUSION WERE THE FACTS THAT INHIBITION WAS MORE PRONOUNCED IN AIR THAN IN AN ATMOSPHERE OF NITROGEN, AND THAT CYSTEINE PREVENTED INHIBITION. EXPERIMENTS PERFORMED WITH THE ADDITION OF VARIOUS GLYCOLYTIC INTERMEDIATES TO BRAIN HOMOGENATES INDICATED THAT THE ENZYME TRIOSE PHOSPHATE DEHYDROGENASE WAS THE PRIMARY SITE OF INHIBITION. THE POTENT INHIBITORY EFFECTS OF QUINONES AND ORTHO AND PARA DIHYDRIC PHENOLS MAKE SUCH COMPOUNDS LIKELY CANDIDATES FOR AN IN VIVO ROLE IN REGULATING ENERGY PRODUCTION FROM GLUCOSE IN THE CENTRAL NERVOUS SYSTEM.

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Pharmacology

MECHANISM OF THE SPECIAL SENSITIVITY OF BRAIN TO GLYCOLYTIC INHIBITION BY QUINONES AND DIHYDRIC PHENOLS. PAUL HOCHSTEIN AND GERALD COHEN (INTR. BY A. GELLHORN). COLUMBIA UNIV., N.Y.

IN A FURTHER STUDY OF THE INHIBITORY ACTION OF QUINONES AND DIHYDRIC PHENOLS (E.G., MENADIONE, EPINEPHRINE, 2,5-DOPA) ON LACTATE PRODUCTION FROM GLUCOSE, IT WAS FOUND THAT BRAIN HOMOGENATES WERE MUCH MORE SENSITIVE TO INHIBITION THAN WERE HOMOGENATES OF OTHER TISSUES. IN ADDITION, INHIBITION WAS SHOWN TO OCCUR AT THE MITOCHONDRIAL LEVEL OF CELLULAR ORGANIZATION WHILETHE DEGREE OF SENSITIVITY TO INHIBITION WAS LINKED TO THE SUPERNATANT (NON-MITOCHONDRIAL) FRACTION OF HOMOGENATES. FOR INSTANCE, MITOCHONDRIA ISOLATED FROM BRAIN COULD BE PROTECTED AGAINST THE INHIBITORY EFFECTS OF 10^{-5}M ADRENOCHROME BY INCUBATION IN LIVER SUPERNATANT; CONVERSELY, LIVER MITOCHONDRIA WHICH IN THEIR NATIVE SUPERNATANT REQUIRE ADRENOCHROME CONCENTRATIONS OF 10^{-3}M TO 10^{-2}M FOR INHIBITION, COULD BE SENSITIZED TO INHIBITION BY INCUBATION IN BRAIN SUPERNATANT. THE PROTECTIVE ACTION OF THE SUPERNATANT FRACTION OF TISSUES OTHER THAN BRAIN APPEARED TO BE RELATED TO THREE FACTORS: THE NON-ENZYMATIC REDUCING CAPACITY OF THE TISSUE, A METABOLICALLY LINKED ENZYMATIC REDUCING CAPACITY, AND THE PRESENCE OF CATALASE.

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Pharmacology

EEG CHANGES FOLLOWING INTRAARTERIAL ADMINISTRATION OF PENTYLENETETRAZOL IN THE RABBIT WITH BASILAR ARTERY TIED. Giovanni F. Ayala,* William G. Van Meter* and Harold E. Himwich. State Research Hospital, Galesburg, Illinois.

Pentylenetetrazol was administered either in the common carotid artery or through the vertebral artery into rabbits with ligated basilar arteries. The dosage necessary to elicit an EEG seizure, injecting in the vertebral artery, was less than the threshold dosage necessary to evoke a similar seizure when the drug was given into the common carotid artery. The same amount of the drug necessary to evoke a seizure, when given in the vertebral artery, administered through the common carotid artery evokes only a low frequency spike and wave pattern or no change at all. However, convulsive activity can be elicited with an injection in the carotid artery if the dosage is increased. Further work is being done in order to determine the possible interrelationships between the two seizures.

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Pharmacology

CEREBRAL FREE AND BOUND SEROTONIN (5HT) AFTER IPRONIAZID (IIH) AND RESERPINE (R). Melvyn I. Gluckman. Vets. Admin. Research Labs. in Neuropsychiatry, Pittsburgh 6, Penna.

Since free 5HT is considered by many to be the physiologically active species of this compound, measurements for it as well as bound were made on the brains of control rats and rats pretreated with IIH or R. Free 5HT is here arbitrarily defined as that remaining in the supernatant following the centrifugation of a homogenate at 100,000g for 2 hours. The pooled brains of 5 rats were used for each determination. Recoveries were checked each time by adding authentic 5HT to one-half of the final preparation used to determine either the free or bound 5HT. Free 5HT was determined directly from the supernatant while bound was determined from a trichloroacetic acid extract of the precipitate using a modification of the method previously described (Fed. Proc. 18:395, 1959). The average of 3 control experiments indicate free and bound 5HT to be present in concentrations of 0.063 ug/gm and 0.38 ug/gm respectively. Following the intraperitoneal injection of IIH (100 mg/kg), free and bound 5HT rose to a peak at 24 hours, 0.26 ug/gm and 0.88 ug/gm respectively. By 72 hours, the bound returned to control level while the free was about 50% higher than control. After R (2.5 mg/kg) both free and bound dropped rapidly being 0.032 ug/gm and 0.061 ug/gm after one hour. At 16 hours neither free nor bound had recovered.

XUM

25

Pharmacology

THE LACK OF CORRELATION BETWEEN THE PSYCHOPHARMACOLOGIC AND ANTICHOLINESTERASE EFFECT OF LSD AND ITS CONGENERS. Elmer K. Zsigmond*, Francis F. Foldes and Vera Foldes*. Dept. of Anesth., Mercy Hospital and Section on Anesth., Dept. of Surgery, University of Pittsburgh School of Medicine, Pittsburgh, Pa.

The inhibitory effect of d-LSD, its pharmacologically inactive isomers (l-LSD, d-iso-LSD) and that of some of its congeners, d-lysergic acid monoethylamide (LAE), BOL and d-lysergic acid morpholide (LSM), on the hydrolysis of acetylcholine by concentrated purified human plasma cholinesterase (Cholase) and by freshly obtained, washed hemolyzed human red cells and on the hydrolysis of acetyl-β-methylcholine and butyrylcholine by human gray matter homogenate was investigated by a manometric method. There was no marked difference between the inhibitory effect of the psychogenic compounds (LSD, LAE and LSM) and that of the non-psychogenic derivatives of lysergic acid (l-LSD, d-iso-LSD and BOL) on cholinesterases. Thus the psychogenic LAE and LSM has a lower inhibitory effect on brain cholinesterase than the non-psychogenic l-LSD, d-iso-LSD or the sedative BOL. (See table.)

Psychogenic compounds:	d-LSD	LAE	LSM
I_{50} values (M/l) for brain ChE:	1.1×10^{-4}	1.0×10^{-2}	6.5×10^{-3}
Non-psychogenic compounds:	l-LSD	d-iso-LSD	BOL
i_{50} values (M/l) for brain ChE:	4.1×10^{-3}	3.0×10^{-4}	1.8×10^{-4}

Supported by U.S.P.H. Grant No. M1425)

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Pharmacology

A COMPARISON OF THE ANTI-CONVULSANT PROPERTIES OF LIDOCAINE AND dl-N-METHYLPIPECOLYL-2,6-XYLIDIDE (CARBOCAINE). C. A. Berry*, J. H. Sanner*, and H. H. Keasling. Dept. of Pharmacology, College of Medicine, State University of Iowa, Iowa City.

In the light of clinical reports of the effectiveness of intravenous lidocaine in the management of status epilepticus in man (Taverner and Bain, Lancet (London), 2:1145, 1958) this activity has been investigated utilizing standard anti-convulsant tests in the mouse. The test procedures used were: maximal electroshock (Swinyard, et al., J. Pharmacol., 106:319, 1952), intravenous Metrazol (Goodman, et al., J. Pharmacol., 108:168, 1953), subcutaneous Metrazol (Swinyard, J. Am. Pharm. A.(Sci. Ed.) 38:201, 1949), and intraperitoneal strychnine (Berger, J. Pharmacol., 112:413, 1954). In addition, inhibition of the motor manifestations and the cortical afterdischarge resulting from repetitive electrical stimulation of the rabbit cortex has been studied. The effects of lidocaine were compared with those of carbocaine, an agent which appears to be orally effective with regard to the elevation of toothpulp threshold in rabbits (Collins, et. al., Fed. Proc., 18:378, 1959). This study indicates that both agents are effective anti-convulsants. (Supported in part by United States Public Health Service grant B-1079).

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Pharmacology

LSD-25 AND BRAIN SEROTONIN IN RESERPINIZED RAT. D.X. Freedman (Intr. by N.J. Giarman) Department of Psychiatry, Yale University School of Medicine, New Haven, Connecticut.

Since whole brain 5-HT tended to be slightly elevated following LSD-25, values in reserpinized brain were determined. 12 to 24 hours after a single dose of reserpine (1 mg/kg) rats were given LSD-25 (0.130 mg/kg) and were sacrificed 20, 60, 120 minutes or 24 hours later. At each of these intervals there was a twofold elevation of brain 5-HT over reserpine controls. Neither metamphetamine nor periods of electroshock to the feet show this effect. A dose effect of LSD-25 is apparent, and with 4 mg/kg reserpine, larger doses of LSD-25 are required. Similar elevations are found with BOL-148 but with doses over 10 times that of LSD-25. That mechanisms governing the status of central amines may be related to the effect as well as the action of LSD-25 is indicated by behavioral studies which show increased impairment and delayed tolerance development with the above dose schedules. (Supported by grant MY3363, NIMH, and The Wyeth Fund.)

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Pharmacology

QUANTIFICATION OF THE EEG ACTIVATION RESPONSE: EFFECTS OF BELLADONNA ALKALOIDS. J. C. Paul-David* and K. R. Unna. Dept. of Pharmacology, Univ. of Ill. Coll. of Med., Chicago, Ill.

A quantification of the EEG activation response (Riehl et al., Psychopharmacologia, 1960) involving integration of frequency and voltage with respect to time was used to evaluate the effectiveness of some of the belladonna alkaloids in depressing the EEG activation response in encéphale isolé cats. Effects of atropine and scopolamine were compared to those of their quaternary N-methyl and N-butyl derivatives. The effects of atropine were also compared to those of its d and l isomers. Graded electrical stimulation of the medial reticular formation was employed to elicit the EEG activation response in the encéphale isolé preparations. The drugs were administered intravenously and dose response curves constructed to determine the minimal effective depressant dose. All quaternary derivatives had a slower onset of action and were significantly less potent than the tertiary alkaloids except N-butyl scopolamine which was equipotent with scopolamine. Both d and l hyoscyamine slowed the EEG rhythm and depressed the activating response. d-hyoscyamine was approximately five times less potent and l-hyoscyamine about five times more potent than atropine. (Supported by the University of Illinois Foundation and Grant B-983 from the National Institutes of Health)

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Pharmacology

THE ACTION OF SOME VERATRUM ALKALOIDS ON ISOLATED FROG NERVE. A. Akcasu*, F. Herr and K. R. Unna. Dept. of Pharmacology, Univ. of Ill. Coll. of Med., Chicago, Ill.

The actions of protoveratrine A and neogermitrine (from veratrum album) were compared with those of veratrine (Schoenocaulon officinale) on desheathed sciatic nerve of frog. Action potentials and membrane potentials were recorded simultaneously using a modification of Stampfli's sucrose bridge method. Protoveratrine A and neogermitrine depolarized the membrane and blocked nerve conduction. Although the depolarization by protoveratrine A and neogermitrine was quantitatively similar to the depolarization by veratrine, the time course of the depolarization was 10-20 times longer than in the case of veratrine. Neogermitrine (0.001%) blocked nerve conduction and depolarized the membrane within 20-25 minutes, if the nerve was not stimulated. When the nerve was stimulated continuously blockade was obtained in 8-10 minutes. The conduction block and the depolarization by neogermitrine was readily reversible by washing with Ringer's solution. The action of protoveratrine A was not reversible. The depolarization and conduction block by neogermitrine and by protoveratrine A, in contrast to that by veratrine, were not reversed by procaine and Ca. (Supported in part by Grant B-973 USPHS.)

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Pharmacology

BIOASSAY OF KANAMYCIN AUDITORY TOXICITY. V. G. Vernier and F. R. Alleva*. Merck Inst. for Therap. Res., West Point, Pa.

Comparative ototoxicity of several antibiotics was assessed using the pinna twitch reflex as a measure of hearing. Daily response to brief Galton whistle (13 KC) stimuli and vestibular capacity was observed in rats before S.C. drug injections. Endpoint for deafness was 3 days absence of pinna twitch. Kanamycin (KM) A yielded a linear dose-response curve with steep slope for log dose versus log mean time onset of deafness. For doses of 600 and 1200 mg./kg., onset occurred at 40 and 13 days. KM C and B yielded parallel curves, the former slightly more potent and the latter 2.5 times as potent as KM A. Neomycin (NM) resulted in a linear dose-response curve of flatter slope. At 100 and 200 mg./kg., onsets were at 42 and 28 days. At highest tolerated doses, streptomycin (SM) and dihydrostreptomycin (DHSM) (480, 800 mg./kg.) caused vestibular deficit but no greater incidence of deafness than in saline controls. A low dose of KM A given concurrently with NM, SM and DHSM shifted the NM dose-response curve to an earlier position, was additive with the lethal effects of SM and DHSM, but not with their ototoxic effects. The latter rats became deaf at a time appropriate only to the KM dosage. Thus, kanamycin and neomycin appear to exert their ototoxic effects in the rat by a similar mode of action which appears to be different from that of streptomycin and dihydrostreptomycin.

31 **Pharmacology**

METHOD FOR SCREENING CENTRALLY-ACTING DRUGS IN THE INTACT RHESUS MONKEY. G.A. Deneau* and M.H. Seevers, University of Michigan Medical Center, Ann Arbor.

The ease with which several types of CNS drugs could be distinguished from each other in the monkey by the uniformity of the patterns of physical signs and behavioral effects produced by the various agents in double blind procedures led us to believe that careful documentation of the effects of all principal classes of CNS drugs would serve as a basis for objective screening of new compounds in this animal. Three dose levels of each drug (low, intermediate and high) are studied. The observed effects are categorized as: autonomic; neurological (including motor); changes in the level of consciousness and alterations in mood. Most of the specific effects can be graded by rigidly defining low, intermediate, severe, and very severe degrees of effect. It can be readily determined whether a new compound belongs to one of the principal classes of CNS drugs or whether it produces a unique pattern of action with potential therapeutic application. Undesirable side effects also become evident during the same observation period. One well trained observer can make complete preliminary evaluation of several drugs in a very short time. (Supported by USPHS Grant MY 2814).

32 **Pharmacology**

PERIPHERAL EFFECTS OF Δ TETRAHYDROCANNABINOL. R. Dagirmanjian* and E.S. Boyd. Dept. of Pharmacology, Univ. of Rochester, N.Y.

The tetrahydrocannabinols comprise one group of the active ingredients in marihuana. A synthesized derivative, dimethyl heptyl pyran (DMHP), was studied in anesthetized cats. The drug was given intravenously as a colloidal suspension in 4% Tween 80-saline. After a dose of 0.1 mg/kg, a gradual, prolonged decrease in blood pressure, gradually returning toward its original level in 2-4 hours, and a decrease in respiratory rate were observed. Relaxation of the gut occurred in approximately 1/2 of the cats. The response of the flexion reflex and linguomandibular reflex were decreased, but little change was seen on the knee jerk. Vagotomy, atropinization, dibenamine, and hexamethonium did not alter the blood pressure effects. Carotid sinus denervation did not alter the respiratory effects. The carotid occlusion pressor response was diminished. Responses to epinephrine and norepinephrine were potentiated, those to isoproterenol diminished. The amplitude of the contractions of the auricles and ventricles was decreased. Transection of the midbrain did not alter the effects seen on blood pressure or the flexion and linguomandibular reflexes, whereas section of the spinal cord at C_1 appeared to abolish these effects. These studies indicate a central locus of action, although a local effect on blood vessels has not been ruled out.

33 **Pharmacology**

EFFECTS OF 'CONVULSANT' ω-AMINO ACIDS DURING CORTICAL MATURATION (CAT). M. W. Carmichael, E. M. Housepian and D. P. Purpura (intr. by H. B. van Dyke.) Depts. of Pharmacol. and Neurosurg., Coll. of Phys. & Surg., N. Y.

Topically applied ε-amino caproic (C_6) and ω-amino caprylic (C_8) acids (1%) augment spontaneous electrocortical activity (SECA) but depress all components of evoked superficial negative cortical responses (SCR) recorded near (1.5-2mm) stimulating electrodes in neonatal cats (< 5d. old). C_8 augments late components of SCR and induces paroxysmal discharges in 6-10d. old animals. C_6 produces similar effects on SCR after the 2nd postnatal week but augmentation of SECA occurs prior to this period. The adult pattern of responsiveness to C_6 and C_8, i.e., rapid augmentation of all components of SCR is established by the 4th week. Distant (4-6mm) SCR's are depressed or unaltered by C_6 and C_8 at a time when near SCR's are augmented. C_6 and C_8 augment hippocampal responses evoked by fimbrial stimulation and induce paroxysmal activity in archicortex at birth. These and other data suggest that inhibitory synaptic pathways develop at different rates in different organizations and that in neocortex those involved in the SCR exert their maximum effects at close range.

34 **Pharmacology**

BEHAVIORAL AND CENTRAL MUSCLE RELAXANT PROPERTIES OF α-METHYL-β-ETHYLVALERAMIDE. Adolph P. Roszkowski* and William M. Govier. McNeil Laboratories, Inc., Philadelphia, Pa.

Pharmacological studies previously carried out in these laboratories (Marsh, D. F., personal communication) suggested that α-methyl-β-ethylvaleramide (McN-X-181) possessed centrally acting skeletal muscle relaxant properties and also affected behavior in animals. This report is an amplification of this incomplete preliminary work. The paralytic dose 50 (PD50) for this agent is 156 mg/kg in mice and 135 mg/kg in rats given i.p. This drug was active in preferentially inhibiting polysynaptic reflex pathways as opposed to monosynaptic pathways in the spinal cat. Doses of 50 to 150 mg/kg orally tranquilized and induced naturally belligerent cats to assume a more gregarious aspect. A taming response was also observed in aggressive monkeys at approximately the same oral dose as in cats. McN-X-181 was active in blocking fighting in paired, preselected naturally belligerent mice subjected to electric suprathreshold foot stimuli. The ED50 here was 95 mg/kg orally, being far below the PD50 for a large therapeutic ratio of 3.69. The 120 min. oral ED50's for blockade of secondary conditioned response, conditioned response, and unconditioned response respectively were 190, 295, and 420 mg/kg.

35 **Pharmacology**

THE EFFECT OF METHYLPHENIDATE ON MOUSE BRAIN FREE CHOLESTEROL. Jon J. Kabara, James T. McLaughlin and Carol A. Riegel (intr. by A. J. Plummer.) Biochemistry Division, Dept. of Chemistry, University of Detroit, Michigan.

Methylphenidate, (Ritalin®) a drug which causes central stimulation, was injected intraperitoneally at two non-toxic dose levels (20 mg/kg and 4 mg/kg) for 6 consecutive days. One hour after the last dose the mice were decapitated and the liver, spleen and brain removed for analysis. The free cholesterol was determined in acetone: alcohol (1:1) extracts of the tissues by precipitation with Tomatine and reaction with the Liebermann-Buchard reagent. Data obtained on 150 mice divided into control and drug groups indicated the following: a) The cholesterol content of the liver, spleen and brain was lowered after drug treatment. Little or no difference however was found in plasma values while the red blood cells of the drug group contained a greater amount of sterol. b) The highest drug dose (20 mg/kg) was more effective in lowering the cholesterol content of the liver than of the brain. c) The greatest decrease of sterol content occurred in the brain with the lower dose (4 mg/kg). Control values of 9.93 ± 0.38 mg/g tissue (wet) were reduced to 7.73 ± 0.36 mg/g tissue.

36 **Pharmacology**

INCREASE IN RAT LIVER TRYPTOPHAN PEROXIDASE AS A RESPONSE TO PITUITARY-ADRENAL STIMULATION BY VARIOUS CENTRALLY ACTING DRUGS. R.P. Maickel* and B.B. Brodie. Natl. Insts. of Health, Bethesda, Md.

The activity of tryptophan peroxidase (TPO) in rat liver has been reported to increase after administration of ACTH, cortisone or hydrocortisone. The present study compares the effect of various centrally acting drugs with physical stimuli (cold and intradermal formaldehyde) on the activity of TPO in rats. Exposure of adult male rats to low temperature (4°C) for 6 hrs results in an increase in TPO activity of about 300%. Intradermal injection of formaldehyde elevates liver TPO by about 150%. Studies with sub-narcotic doses of barbiturates suggested that the increase in TPO activity was related to the stability of the barbiturate in the body. For example, barbital (30 mg/kg I.P.) and phenobarbital (20 mg/kg I.P.) disappear slowly and elevate TPO about 100%. Hexobarbital (20 mg/kg I.P.) which is metabolized rapidly does not produce a significant elevation of TPO. Chlorpromazine stimulates TPO by 150% in doses of 10 mg/kg, I.P., while the structurally related compound, imipramine (Tofranil), does not change TPO levels in doses up to 40 mg/kg. Iproniazid (80 mg/kg I.P.) increases TPO by 100%. No increase in TPO activity could be found after application of stimuli to hypophysectomized animals, thus implicating the pituitary as the mediator for this response.

XUM

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Pharmacology

SOME BIOCHEMICAL EFFECTS OF RESERPINE MEDIATED BY THE PITUITARY. E.O. Westermann*, R.P. Maickel* and B.B. Brodie. Natl. Insts. of Health, Bethesda, Md.

The profound pharmacological effects of reserpine on hypothalamic centers suggests that this drug might affect the control of pituitary secretion. We have studied the effect of reserpine on the pituitary-adrenal system. Despite the "anti-stress" nature of reserpine, and reports that small doses can inhibit the response of the pituitary-adrenal axis to stressfull stimuli, we have found that relatively large doses of the drug elicit a marked pituitary-adrenal response in rats characterized by depletion of adrenal ascorbic acid, an increased plasma level of free fatty acids and corticosterone and an increased liver tryptophan peroxidase activity. Significant effects were observed in doses of reserpine (about 0.4 mg/kg I.V.) that lowered brain serotonin and norepinephrine by 50%. Doses (1.0 mg/kg I.V.) that completely depleted brain amines elicited maximal effects which were not increased by doses up to 5.0 mg/kg I.V. None of these responses were produced in hypophysectomized animals. In confirmation of results of Kitay et al. (Endocrinology 65: 548, 1959), we found that high doses of reserpine produced considerable depletion of pituitary ACTH. Rats pretreated with dibenamine (10 mg/kg I.P.) did not show the pituitary-adrenal response to reserpine.

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Pharmacology

DEPLETION OF BRAIN NOREPINEPHRINE BY RESERPINE WITHOUT PRODUCING SEDATION. F. Barbara Orlans*, Fridolin Sulser* and B.B. Brodie, Natl. Insts. of Health, Bethesda, Md.

It is difficult to associate tranquilizing action of reserpine to either brain norepinephrine or serotonin since they are released to the same degree. Moreover, despite reports to the contrary, the sedative effects of neither raunescine nor benzoquinolizines are associated with a selective loss of brain norepinephrine. However, reserpine administered to rats or rabbits stressed by cold-exposure, does not induce sedation nor appreciably lower brain serotonin, but markedly lowers brain norepinephrine. A close association between appearance of sedation and the release of brain serotonin was shown by experiments in which rats were exposed 4 hours to cold, given reserpine and then brought to room temperature. The levels of brain serotonin now declined slowly and sedation appeared only when the serotonin level had declined by about 50%. Preliminary experiments on the mechanism of the stress-induced prevention of brain serotonin release suggest little or no change in monoamine oxidase, in 5-hydroxytryptophane decarboxylase, or in serotonin binding.

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Pharmacology

ANTAGONISTIC ACTIONS OF IMIPRAMINE (TOFRANIL) AND RESERPINE ON CENTRAL NERVOUS SYSTEM. F. Sulser*, J. Watts* and B.B. Brodie, Natl. Insts. of Health, Bethesda, Md.

Imipramine used clinically as an antidepressant is not a monoamine oxidase inhibitor. It does not elicit excitation in normal man or animals, but acts only in abnormal (depressed) states. Domenjoz and Theobald (Arch. Int. Pharm. 120: 450, 1959) noted that imipramine blocks the potentiating action of reserpine on a hypnotic agent. This prompted us to investigate whether reserpine-induced depression might serve as a model to study the antidepressant action of imipramine. Reserpine (2.5 mg/kg I.P.) given to rats prolongs the narcosis elicited by alcohol (3 g/kg I.P.) from the control time of 5 min. to about 120 min. In animals pretreated by imipramine (25 mg/kg I.P.), 3 to 6 hrs before giving reserpine, the potentiation is almost completely blocked. In contrast, imipramine does not inhibit the alcohol potentiation induced by chlorpromazine. Imipramine-treated animals do not show ptosis after reserpine administration and seem more active than control animals receiving reserpine alone. Imipramine does not prevent release of brain serotonin by reserpine. Since imipramine exerts its anti-reserpine effects in hypophysectomized rats, it does not act like cold-stress, which prevents both reserpine sedation and release of serotonin.

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Pharmacology

METHYLATION IN THE METABOLISM OF (-)-NICOTINE. LENNOX B. TURNBULL,* EDWARD R. BOWMAN,* AND HERBERT MCKENNIS, JR., MEDICAL COLLEGE OF VIRGINIA, RICHMOND, VA.

IN THE METABOLISM OF (-)-NICOTINE IN THE DOG A WIDE VARIETY OF OXIDATIVE CHANGES OCCUR IN THE PYRROLIDINE RING. SINCE MOST OF THE KNOWN METABOLITES ARISING IN THIS PROCESS ARE FORMED FROM THE INTERMEDIATE (-)-COTININE, AN INVESTIGATION OF N-METHYLATION OF THE PYRIDINE RING OF COTININE APPEARED DESIRABLE. SYNTHETIC COTININE METHOIODIDE WAS PREPARED BY REACTION OF COTININE AND METHYL IODIDE. FOLLOWING ADMINISTRATION (-)-COTININE THE URINE OF DOGS CONTAINED A QUATERNARY COMPOUND WHICH WAS RETAINED BY DOWEX 50 (H^+). THE ACID-ELUATE OF THIS RESIN GAVE A CRYSTALLINE REINECKATE WHICH WAS CONVERTED TO AN IODIDE, M.P. 163-164°, IDENTICAL TO THE SYNTHETIC PRODUCT. FOLLOWING ADMINISTRATION OF (-)-NICOTINE, DOG URINE CONTAINS SEVERAL COMPONENTS REACTING WITH DRAGENDORFF REAGENT. A MAJOR COMPONENT CORRESPONDS IN R_F VALUE TO NICOTINE ISOMETHOIODIDE UPON PAPER CHROMOTOGRAPHY IN SEVERAL SOLVENT SYSTEMS. (AIDED BY GRANTS FROM THE TOBACCO INDUSTRY COMMITTEE AND THE AMERICAN TOBACCO CO.)

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Pharmacology

THE USE OF AMINOPHENYLPYRIDONE AS AN ATARACTIC AGENT IN OUTPATIENTS. John H. Nodine,* Tibor Bodi*, Howard A. Levy*, Peter E. Siegler* and John H. Moyer. Hahnemann Med. Coll. and Hosp., Philadelphia, Pa.

A group of 44 patients were selected after a complete history and physical examination, laboratory examinations and indicated consultations. These patients, who had significant psychiatric or psychosomatic symptomatology, were given aminophenylpyridone in daily doses ranging from 200 to 600 mg. for periods of 2 to 15 weeks. Highly gratifying therapeutic response occurred in 36 of the 44 patients and definite improvement was found in an additional 5 cases. It seemed especially useful in 13 cases of tension headache. Aminophenylpyridone was remarkably free from side effects both clinically and in the toxicology laboratory. It is a mild to moderately potent ataractic agent which is suitable for outpatient administration.

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Nutrition

THE ACTION OF CHLORPROMAZINE HYDROCHLORIDE (SKF NO. 2601-A) ON YEAST HEXOKINASE. Thomas Masurat,* Edward G. Rice,* John F. Herndon and Samuel M. Greenberg. Res. & Dev. Div., Smith Kline & French Labs., Philadelphia, Pa.

It was observed in the course of earlier studies performed in this laboratory that the in vivo administration of chlorpromazine hydrochloride decreased the rate of pyridoxal-5-phosphate formation from ATP and pyridoxal in brain homogenates from normal and vitamin B_6-deficient rats (Fed. Proc. 18: 536, 1958). These results were interpreted to indicate that chlorpromazine prevented the transfer of phosphate from ATP to pyridoxal by inhibiting the action of pyridoxal kinase. In order to further investigate the effect of chlorpromazine on substrate phosphorylations, we studied the effect of the drug on the yeast hexokinase reaction. Within the range of the drug concentrations investigated (8.5×10^{-6} M to 6.8×10^{-5} M), hexokinase activity was either greatly activated or inhibited by chlorpromazine, depending on the level of magnesium-ATP present in the system. This biphasic action of chlorpromazine suggests that one of the actions of the drug in vivo may be to influence the rate of utilization of ATP.

175. NEUROPHARMACOLOGY– Analeptics

1 *Pharmacology*

A STUDY OF SOME CENTRAL STIMULANTS IN MICE. L. B. Witkin, M. Maggio, E. O'Keefe and F. Galdi (intr. by A. J. Plummer) Research Dept., CIBA Pharmaceutical Products Inc., Summit, N.J.

Certain central stimulants of which methylphenidate and amphetamine are typical are about ten times more toxic in mice subjected to the stress of crowding. In the evaluation of analgesic activity by the tail flick method in which animals are confined in a container which rigidly restricts their movements (stress?) these same compounds markedly potentiate the analgesic effects of morphine in comparatively low doses. Reserpine causes a marked antagonism to morphine analgesia measured in this way. Another group of central stimulants of which metrazol is typical are as toxic when the animals are crowded as when they are isolated. Furthermore, these compounds conspicuously lack the ability to potentiate analgesics. On the basis of this and other data to be presented it is proposed that animals under stress have a vastly higher level of some endogenously elaborated neurohormone which is itself an excitant. This material is antagonized by reserpine and potentiated by methylphenidate-like agents but not by compounds like metrazol.

2 *Pharmacology*

A NEW STIMULANT. 5-PHENYL-2-IMINO-4-OXO OXAZOLIDINE (FWH-352). J.D. McColl and W.B. Rice*. Research Laboratories, Frank W. Horner Limited, Montreal.

FWH - 352, a non-convulsant stimulant of low toxicity, has a maximal effect on spontaneous motor activity in mice following IP doses of 20 - 40 mg/kg. Peak effect occurred between 60 - 90 minutes with increased activity evident for several hours. This stimulant activity was intermediate between caffeine and amphetamine. Against reserpine-induced motor depression this compound was antagonistic at doses in excess of 80 mg/kg. Caffeine and amphetamine under similar conditions were effective at doses which were maximally active for motor stimulation in normal animals. Hexobarbital narcosis was prolonged but this effect was considerably less than that observed with iproniazid. FWH - 352 antagonized alcohol-induced hypnosis. The compound had less amino oxidase inhibitory activity than iproniazid and resembled amphetamine in its mode of action on the CNS.

3 *Pharmacology*

INFLUENCE OF BRAIN LESIONS ON SENSITIVITY TO AMPHETAMINE AS MEASURED BY SPONTANEOUS LOCOMOTOR ACTIVITY. Martin W. Adler (intr. by M. E. Jarvik). Dept. of Pharmacology, Albert Einstein Coll. of Med., New York City.

Very few studies involving changes in sensitivity to pharmacological agents after chronic brain damage have been reported. An increase in the number of amphetamine-induced convulsions after brain lesions in monkeys has been reported (Blum, Blum and Chow, Arch. Neurol. Psychiat. 64:685, 1950). In the present study, activity in a stationary rectangular cage was measured by means of photoelectric cells. A dose of amphetamine which caused an increase in activity in normal animals, 2.5 mg/kg, was used. Each of three groups of male albino rats was subjected to bilateral lesions of frontal cortex, posterior cortex, or caudate nucleus. Frontal animals showed a significant increase in sensitivity to amphetamine ($p=.001$) as compared to normal animals, as did animals with posterior lesions ($p<.01$). Caudate animals showed a slight increase which was not significant. These changes were persistent. These findings indicate that brain-damaged individuals may have altered sensitivity to drugs and that such differences may vary according to the locus of the injury.

4 *Pharmacology*

INDOKLON AS AN INTRAVENOUS CONVULSANT AGENT. Alfred S. C. Ling*, John C. Krantz, Jr. and Albert A. Kurland*. Univ. of Md. Schl. of Med. and Spring Grove State Hosp., Baltimore, Md.

The use of Indoklon as a volatile, inhalant convulsant has been described (J. Pharmacol. Exper. Therap. 121:362,1957; J. Amer. Med. Assn. 166:1555,1958). An Indoklon solution suitable for i.v. injection, the pharmacological response of animals to i.v. Indoklon, and preliminary clinical trials were studied. A 5 vol.% Indoklon was prepared using polyethylene glycol 300 and water. Subsequently 95% ethyl alcohol was added. The solution was stable upon autoclaving at 15 lbs. pressure for 25 mins. An i.v. dose of 0.2 ml/kg consistently induced convulsions in monkeys and dogs pretreated with atropine sulfate and succinylcholine chloride. EEG tracings showed cortical seizure activity. Subchronic toxicity studies on dogs indicated no abnormal hematological changes except for a slight hemoconcentration. The average latency was 10 secs. The average convulsion lasted 68 secs. The average recovery time, i.e. from onset of convulsion to locomotion, was 140 secs. There were 1-2 convulsions/injection. Two dogs, given massive doses of 1.0 ml/kg i.v., suffered no untoward effects. Five patients were each given 0.2 ml/kg i.v. of 5% Indoklon. This dose, often less, never failed to evoke convulsion in man. The response was similar to that induced by the inhalation technic. There was no tissue damage at the site of injection. (Supported by grants from N.I.M.H. and the Ohio Chem. & Surg. Equip. Co.)

5 *Pharmacology*

ACTION OF METHYLATED XANTHINES ON ELECTRICALLY EVOKED AFTER-DISCHARGE IN THE NEURONALLY ISOLATED DOG CEREBRAL CORTEX. Ajit Maiti* and Edward F. Domino, Dept. of Pharmacology, University of Michigan, Ann Arbor.

Although the methylated xanthines have long been considered to act on the cerebral cortex little direct neuropharmacological data have been available to support this hypothesis. Therefore the effects of caffeine, theophylline and theobromine were determined on the evoked electrical activity of the neuronally isolated cerebral cortex preparation. A portion of the suprasylvian gyrus with an intact pial circulation was isolated neuronally in mongrel dogs anesthetized with halothane. After completion of the surgery the halothane was discontinued and the animals were given decamethonium and placed on artificial respiration. The duration of afterdischarge evoked by repetitive electrical stimulation and the spontaneous EEG were recorded at 5 minute intervals from the isolated and surrounding intact cerebral cortex. The methylated xanthines were given intravenously in doses of 0.25 to 8 mg/kg calculated as base. Caffeine was most effective in prolonging cortical afterdischarge. The peak of effect usually occurred within 15 minutes and lasted approximately 1 hour. Theophylline was less potent than caffeine. Theobromine in doses up to 4 mg/kg had little to no effect in prolonging cortical afterdischarge.
(Supported in part by grant MY 2653 C1 USPHS).

176. NEUROPHARMACOLOGY– Analgesics

1 *Physiology*

INHIBITION OF THE RELEASE OF INTRACELLULAR POTASSIUM BY NON-NARCOTIC ANALGESIC AGENTS. Fred B. Benjamin (With the technical assistance of V. Monteleone.) From the Biological Research Division of the Colgate Palmolive Co., New Brunswick, N.J. (Present address: Life Science and Space Development Division, Republic Aviation Corporation, Farmingdale, L.I., N.Y.)

Intestinal strips of guinea pigs (two and a half inches long) are suspended in K free Tyrode solution. This causes release of K into the bathing fluid. The amount of K released increases with the distention pressure and it increases in a caudal direction with the anatomical position of the strip. In the presence of non-narcotic analgesic agents (acetanilid, phenacetin, aminopyrine) in pharmacological concentrations the post-distention K concentration of the bathing fluid is decreased and that of the intestinal wall is increased. The release of K on exposing washed human erythrocytes to cold (7°C) is similarly inhibited by the analgesic agents.

2 *Physiology*

ON THE ANTAGONISM BETWEEN MORPHINE AND LEVALLORPHAN. Joseph H. Gans and Arthur L. Aronson (intr. by H. H. Dukes.) Cornell Univ., Ithaca, N. Y.

Intravenous morphine administration (3.3 mg/Kg) to dogs anesthetized with pentobarbital sodium was associated with hypoventilation, decreased oxygen consumption and decreased blood pressure. Levallorphan administration (1 mg/37.5 mg of morphine) resulted in prompt reversal of the respiratory depression and in an increase in blood pressure. Repeating the administration of levallorphan further augmented respiration; hyperventilation occurred in dogs having low arterial CO_2 content (< 42 vol.%). Levallorphan prevented the increases in arterial CO_2 content and the decreases in oxygen consumption and blood pressure which typically accompany morphine administration, but did not prevent a decrease in rate of change of minute volume. Physostigmine (0.05 mg/Kg) neither overcame morphine depression nor augmented levallorphan antagonism. Larger doses of physostigmine (0.10 mg/Kg) produced respiratory arrest; atropine restored respiratory activity to control levels or to levels anticipated by prior administration of morphine and levallorphan. The antagonism between morphine and levallorphan appears to be competitive but the data do not eliminate the possibility of non-equilibrium antagonism; changes in the rate of activity of the cholinergic system are not directly involved.

3 *Pharmacology*

TOLERANCE TO MORPHINE - I. EFFECTS ON CATECHOL AMINES IN BRAIN AND ADRENAL GLANDS. E. W. Maynert and G. I. Klingman*. Dept. of Pharmacol., Johns Hopkins School of Med., Baltimore, Md.

The acute administration of morphine sulfate (M) to rats (60-150 mg/kg) or dogs (200 mg/kg) reduced the concentration of brain norepinephrine (Br-N) and of adrenal catechol amines (Ad-CA) a situation similar to that found by Vogt in cats (J. Physiol. 123:451, 1954). Rats, dogs and rabbits made tolerant to M (60-200 mg/kg) had normal or supranormal Br-N and Ad-CA. After JB-516 (8 mg/kg) M-tolerant rats showed a larger increase in Br-N than did control rats. In M-tolerant dogs, nalorphine (5 mg/kg) caused hyperthermia, an excitatory withdrawal syndrome and a fall in Br-N and Ad-CA, regardless of the occurence of convulsions. In tolerant rats nalorphine (10-20 mg/kg) caused hypothermia and sedation but no changes in Br-N or Ad-CA. Tolerant rabbits exhibited a mild excitatory syndrome after nalorphine, but only occasional animals which convulsed had lowered Br-N. (Support by Grant B-1402, U.S.P.H.S.)

4 *Pharmacology*

TOLERANCE TO MORPHINE - II. EFFECTS ON CATECHOL AMINES IN HEART, INTESTINE AND SPLEEN. G. I. Klingman* and E. W. Maynert. Dept. of Pharmacol., Johns Hopkins School of Med., Baltimore, Maryland.

Rats tolerant to a daily dose of 200 mg/kg of morphine sulfate exhibited increased excitability but had heart rates 25% lower than controls. The concentration of norepinephrine in the hearts of tolerant rats sacrificed 5 or 29 hours after the last dose of morphine was 30% lower than in controls. Norepinephrine in the small intestine was depressed to a similar extent (35%). The spleens of tolerant rats were smaller than those of controls and contained 35% less norepinephrine. The administration of nalorphine (10-20 mg/kg) to tolerant rats caused diarrhea and sedation; heart rate was increased slightly. After nalorphine heart and intestinal norepinephrine of tolerant rats returned practically to the levels of control rats in 5 hours. Splenic norepinephrine also appeared to rise. (Support by Grant B-1402, U. S. P. H. S.)

5 *Pharmacology*

THE EFFECTS OF CARISOPRODOL ON CNS ACTIVITY. Milton Kletzkin. (Intro. by F.M.Berger.) Wallace Laboratories, New Brunswick, N.J.

Carisoprodol which shows both muscle-relaxant and analgesic properties has been studied in unanesthetized, immobilized cats. Five mg/kg of carisoprodol, i.v., reduced the frequency of the spontaneous EEG from 25-35 cps to about 12-18 cps at a higher amplitude. A 10 mg/kg dose produced a "sleepy" EEG pattern. Such a dose has no behavioral effects. As additional amounts of the drug were administered, the frequency continued to decline and the voltage to increase until after about 60 mg/kg only occasional deflections appeared against an otherwise inactive record. EEG activation produced by stimulation of the sciatic nerve, mesencephalic reticular formation, or diffuse thalamic system (N.centralis lateralis, or N. reticularis) was depressed by 10 mg/kg of carisoprodol. Recruiting responses recorded during low frequency stimulation of these diffuse thalamic nuclei were enhanced by 10-20 mg/kg. The durations of hippocampal afterdischarges elicited by stimulation of the fornix were not shortened by carisoprodol in doses up to 40 mg/kg. These effects contrast sharply with those produced by meprobamate but resemble in various respects the actions of morphine, pentobarbital and atropine.

6 *Pharmacology*

EFFECT OF ADMINISTRATION OF L-THYROXIN ON N-DEMETHYLATING ACTIVITY IN NORMAL AND MORPHINE-TREATED RATS. Joseph Cochin and Louis Sokoloff.* National Institutes of Health, Bethesda, Md.

Recent studies have indicated that L-thyroxin (Tx) may accelerate the rate of protein synthesis. Following chronic administration of narcotic drugs to rats, profound depression of demethylation of narcotic drug substrates by liver preparations is observed; this activity returns to normal 8-14 days after cessation of drug treatment. Since this recovery may reflect enzyme resynthesis, the effect of Tx on the recovery of N-demethylating activity was investigated. Forty-four male rats were divided into four groups and treated as follows: Group A, morphine (M) twice daily for 30 days in increasing amounts to a maximum of 200 mg/kg per day; Group B, M as in A, Tx 90γ per day for 7 days prior to withdrawal of M, and up to the day the animals were sacrificed; Group C, Tx alone, same dose and time schedule as in B; Group D, control. Animals from each group were sacrificed on days 1, 4 and 8 after termination of M administration. Enzyme activity in percentage of value obtained from controls was: day 1, A 7%, B 3%, C 101%; day 4, A 13%, B 18%, C 66%; day 8, A 71%, B 24%, C 54%. The results show that M and Tx depress N-demethylase activity separately and together and that the recovery of this activity in M-treated animals subjected to Tx is delayed significantly rather than accelerated.

7 *Pharmacology*

EFFECT OF CARISOPRODOL AND OTHER ANALGESICS UPON TOOTH PULP PAIN IN HUMANS. S. Margolin. Wallace Laboratories, New Brunswick, N.J.

A neural structure consisting only of pain endings, such as found in human tooth pulp, should critically determine the analgesic properties of drugs. Consistent responses obtained by a high frequency stimulator, as employed in vitality tests of human tooth pulp, furthered the practicability of analgesic evaluation based upon this neuroanatomical structure. By means of a "Vitalometer" providing a current generator with over 3 million ohms resistance and constant current of 4 microamperes, the right upper incisor was stimulated for 2 seconds, every 5 to 10 seconds, at increasing voltages until pain occurred. Nineteen men and women 22 to 52 years of age participated. Neither subjects nor observer knew whether drug or placebo were given. Pain thresholds were determined just before the drugs were given orally; again at 1 and 2 hours thereafter. Six days elapsed between successive trials. Compared to placebo, carisoprodol (700 mg.) significantly ($P = 0.05$) raised pain threshold at 1 hour (26%) and 2 hours (52%). Codeine phosphate (30 mg.) significantly elevated threshold at 1 hour (29%) and 2 hours (27%). Acetylsalicylic acid (600 mg.) did not significantly increase threshold (9%). Since this experiment employed an end-point not requiring muscular reaction, carisoprodol induced analgesic in humans independent of its muscle relaxant action.

8 *Pharmacology*

AN EVALUATION OF ANALGETIC AGENTS AS DEMONSTRATED BY THE TECHNIQUE OF RANDALL AND SELITTO AND THE D'AMOUR-SMITH RAT TAIL-FLICK METHOD. L.C. Greene*, S.A. Ferguson*, and H. Wendel. Smith Kline & French Laboratories, Philadelphia, Pennsylvania.

A new method for measuring analgesic activity has been developed and reported by Randall and Selitto (Arch. int. pharmacodyn, 1957, CXI, 409). This method is based on the principle that inflammation increases the sensitivity to pain and that this increased sensitivity is susceptible to modification by analgesics of both the narcotic and non-narcotic type. While the radiant heat methods are applicable for the evaluation of the more potent analgetics, they leave a lot to be desired in the measurement of analgesics of the aminopyrine, sodium salicylate, and phenylbutazone type compounds. The present communication is concerned with our experience with the two techniques. Although the sensitivity of these two techniques differs, the dose response curves obtained are qualitatively similar. We were unable to reproduce the dose response curves for aminopyrine and sodium salicylate previously reported by Randall and Selitto. Higher doses of both of these compounds produced side effects such as excitation and/or decrease in spontaneous motor activity resulting in a questionable endpoint. The Hoffmann-LaRoche technique appears to have some merit in the evaluation of the weaker, non-narcotic analgetic agents over the currently available thermal radiation methods.

9 *Pharmacology*

CROSS-TOLERANCE TO ANALGESIA BETWEEN PHENAZOCINE AND OTHER ANALGESICS. Charles B. Carter* and Norman A. David. Dept. of Pharmacology, Univ. of Oregon Medical School, Portland, Ore.

Daily s.c. injections and the D'Amour-Smith radiant heat method were used to determine the percentage increase in the average of the pain reaction time (analgesic response, A.R.) in rats. With 2 mg/kg morphine sulfate given 6 days a week for 9 weeks the A.R. decreased from 72% to 18%, with 0.1 mg/kg phenazocine from 55% to 32% and with 10 mg/kg meperidine from 61% to 36%. During withdrawal the A.R. returned to initial values in 2 weeks for morphine and within 1 week for phenazocine and meperidine. In cross-tolerance studies the A.R. was 36% and 39% in 2 groups of rats given 0.15 mg/kg phenazocine for 63 days; when 2 mg/kg morphine was substituted on the 64th. day in the first group the A.R. was 46% and in the group continued on phenazocine, 41%. Three groups of rats given 0.15 mg/kg phenazocine for 21 days showed decreases in the A.R. from 74% to 50%, from 79% to 54% and from 73% to 47%. With continuation of phenazocine to 42 days in 1 group the A.R. was 36% and with cross-over to 2 mg/kg morphine in the second group, 23% and with 12.5 mg/kg meperidine, 38% on the 42nd. day. Changing the order of drug administration and using other analgesics did not alter the pattern of continuing decrease in the A.R. but allowed comparisons to be made in the rate of the development of tolerance and degree of cross-tolerance.

10 *Pharmacology*

EFFECTS OF ANALGESICS ON AUDIOGENIC SEIZURES IN MICE. E. Moffat*, R. F. Krueger*, R. O. Pfeiffer* and D. M. Green. Product Planning Division, Grove Laboratories Incorporated, St. Louis, Mo.

Many of today's well-known tranquilizers are effective in protecting against audiogenic seizures in mice and rats. Susceptibility to audiogenic seizures was investigated in nine strains of mice using the procedure described by Plotnikoff and Green (J. Pharm. & Exp. Therap., 119:2, 1957). Susceptibility of weakly-responding strains was not increased by withholding food or water, oral administration of 50 mg./Kg. of caffeine, or use of juvenile mice. One commercially-available carcinogenic strain (now designated "Audiogenic" strain) was many times more susceptible than the best of the other strains. From 50 to 95% responded with an easily distinguishable syndrome which is remarkably permanent and reproducible upon hourly or daily retrial. Using chlorpromazine and meprobamate as reference standards, a number of analgesics were studied for protective potency. Aspirin and N-acetyl-p-aminophenol were ineffective while the oral 50% protective dose (PD_{50}) of salicylamide and phenacetin were 200 and 240 mg./Kg., respectively. Among other drugs studied glyceryl guaiacolate was active (PD_{50}=700 mg./Kg.) while adiphenine and phenyltoloxamine were ineffective. A combination of drugs individually inactive, N-acetyl-p-aminophenol, adiphenine and phenyltoloxamine, nevertheless, established a PD_{50} of 443 mg./Kg. in a 10:1:1.17 mixture, suggestive of true synergism.

11 *Pharmacology*

EFFECTS OF ANALGESICS ON THE MESCALINE SCRATCH RESPONSE IN MICE R. F. Krueger*, D. E. Lauck*, E. Moffat*, and D. M. Green. Product Planning Division, Grove Laboratories Incorporated, St. Louis, Mo.

The scratch response of mice induced by the psychotomimetic agent, mescaline, was studied in four strains of male mice. The response of dba mice was three times that obtained from the C.F.W., Swiss and Audiogenic strains. Single oral doses of mescaline resulted in subsequent refractiveness of the scratch response to further doses of mescaline for periods up to several weeks. Drugs and environmental factors (e.g. darkness, noise) which increase mouse motor activity decrease the scratch response. Drugs were evaluated in terms of 50% inhibition of the scratch response (PD_{50}). On oral administration the PD_{50} of chlorpromazine, the reference standard, was 0.58 mg./Kg. The PD_{50}s of aspirin, salicylamide and phenacetin were 108, 182, and 205 mg./Kg., respectively. Among other drugs which were active were adiphenine, 158 mg./Kg.; glyceryl guaiacolate, 155 mg./Kg.; and phenyltoloxamine, 9 mg./Kg. Meprobamate was ineffective and at low doses even enhanced the scratch response.

12 *Pharmacology*

THE DISTRIBUTION OF TRITIUM-LABELED NALORPHINE IN THE CENTRAL NERVOUS SYSTEM OF DOGS. C.C. Hug, Jr.* and L.A. Woods. Dept. of Pharmacology, Univ. of Michigan Med. School, Ann Arbor.

Tritium-labeled nalorphine (prepared as reported at the Fall Pharmacology Meeting, Miami, Fla., 1959) was estimated as free drug in tissue samples weighing 0.1-1 gm. A volume of homogenate prepared in 0.5 N HCl was mixed with 0.5 mg "cold" nalorphine, neutralized, buffered at pH 9, and extracted with ethylene dichloride containing 10% n-amyl alcohol. An aliquot of the organic extract was evaporated to dryness and the residue dissolved in 0.8 ml of n-amyl alcohol. After the addition of phosphor solution, the radioactivity was determined in a Packard Tri-Carb spectrometer. Nalorphine was injected subcut. in doses of 2 mg/kg (calcd. as free base) into mongrel dogs weighing 8-10 kg. After suitable intervals, the dogs were sacrificed with a rapid I.V. injection of thiopental and the cardiac ventricles incised. The brain was perfused with saline through the carotid artery. Areas of the CNS were removed and selected samples separated into gray and white components. Gray tissue attained a peak conc. of 1340 millimicrograms (mμgm)/gm in 30-60 minutes and the level was 60 mμgm/gm at 4 hours. A maximum conc. of 1140 mμgm/gm was found in white tissue at 30-60 minutes; at 4 hours the level was 150 mμgm/gm. Spinal cord gray and white levels were variable, but in the range of cortical conc. (Supported in part by Grant B-625 USPHS).

13 *Pharmacology*

DISTRIBUTION OF FREE N-C^{14}-METHYL LABELED MORPHINE IN THE CNS OF DOGS. S.J. Mule* and L.A. Woods. Dept. of Pharmacology, Univ. of Michigan, Ann Arbor.

Mongrel dogs weighing 5-10 kg were injected subcut. with 2 mg/kg (calcd. as free base) of the labeled morphine. The animals were sacrificed by rapid I.V. injection of thiopental and incision of the cardiac ventricles at intervals of 0.6-16 hours. The brain was perfused with saline via the carotid artery. Tissue samples of selected areas of the brain and spinal cord weighing approximately 0.1-1 gm were homogenized in 0.5 N HCl. An aliquot was mixed with 0.5 mg non-labeled morphine carrier, neutralized, buffered to pH 9, and extracted with ethylene dichloride containing 10% ml n-amyl alcohol. The extracts were evaporated to dryness. The residue was dissolved in 0.8 ml n-amyl alcohol and mixed with 10 ml of phosphor solution. Radioactivity was estimated by internal liquid scintillation. The cerebral cortical conc. increased rapidly for 0.6 hour and then remained relatively constant through 8 hours. The apparent maximum values of 230 millimicrogram (mμg)/g and 125 mμg/g occurred at 4 hours in gray and white areas, respectively. At 16 hours the conc. in both gray and white areas was about 50 mμg/g. Analgesia persisted for 12 hours. (Supported by USPHS Grant B-625).

XUM

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Pharmacology

THE DISTRIBUTION OF FREE N-C14-METHYL LABELED LEVORPHANOL IN THE CNS OF NON-TOLERANT DOGS, NON-TOLERANT AND TOLERANT MONKEYS. K.D. Wuepper* and L.A. Woods. Dept. of Pharmacology, Univ. of Michigan Med. School, Ann Arbor.

After subcut. injection of 2 mg/kg (calcd. as free base) of labeled levorphanol, non-tolerant dogs and monkeys were sacrificed at intervals up to one hour with a rapid I.V. injection of thiopental. Each brain was perfused through the carotid with 0.9% saline. Tolerant monkeys, stabilized on non-labeled levorphanol injected subcut. every six hours, were given 16 consecutive injections of radioactive drug on the same dosage schedule and sacrificed, as above, one hour after the last injection. Samples of the CNS were homogenized in 0.5 N HCl, neutralized, and estimated for the free drug using the method described previously (J. Pharmacol. & Exper. Therap., 124: 1, 1958), but modified for counting by internal liquid scintillation. The residue, after evaporation of the ethylene dichloride extract, was dissolved in 0.8 ml n-amyl alcohol and 10 ml of phosphor were added. Grey and white cortical samples of the non-tolerant dog at one hour averaged 2300 millimicrograms (mμg)/g and 1450 mμg/g, respectively. The mean values of grey (ca. 1900 mμg/g) and white (ca. 1400 mμg/g) cortical samples in the non-tolerant and tolerant monkey at one hour were not significantly different. (Supported in part by USPHS Grant B-525).

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Pharmacology

CENTRAL AND PERIPHERAL ACTION OF ANALGESICS. Christian Braun*, Frank Guzman* and Robert K. S. Lim. Med. Sci. Res. Lab., Miles Laboratories, Inc., Elkhart, Indiana.

It is known that narcotic and non-narcotic analgesics lower the temperature of the yeast inflamed rat's foot, and that this effect is related to their analgetic effect in that pari passu the pressure threshold for vocalization, foot withdrawal and struggle is raised. It was therefore of interest to determine whether the local temperature change was due to peripheral or central action. When one hind foot was denervated, temperature recordings showed that morphine and aminopyrine lowered the local temperature in the innervated foot only, while acetylsalicylic acid and N-acetyl-p-aminophenol lowered the local temperature in both innervated and denervated foot. Thus it appears that the analgesic action of morphine and aminopyrine is central, while that of acetylsalicylic acid and N-acetyl-p-aminophenol is peripheral. Other evidences in support of this conclusion will be presented.

16

Pharmacology

MORPHINE-NALORPHINE ALTERATION OF TOOTH PULP THRESHOLDS OF DOGS IN THE ALERT AND DROWSY STATE. Jane E. Heng* and Edward F. Domino, Dept. of Pharmacology, Univ. of Mich., Ann Arbor.

Inasmuch as morphine causes drowsiness, it was important to know if the elevation of tooth pulp thresholds produced by morphine was greater than that due to drowsiness alone. Six mongrel dogs were chronically implanted with bipolar electrodes in the upper canines. Each tooth was usually stimulated repetitively at 100 cps and with single shocks every 4 seconds for one minute until a definite response threshold was obtained in both the alert and drowsy states. When stable thresholds were determined, 2 mg/kg of morphine was given and antagonized by 1 mg/kg of nalorphine. Both agents were given intravenously. Repetitive stimulation consistently elicited more reproducible responses at a much lower voltage than single shocks. After morphine single shock thresholds were so markedly elevated that they could not be accurately determined. Therefore the effects of morphine on the threshold to repetitive stimulation were compared to those in the alert and drowsy state. Tooth pulp thresholds in the following states were significantly different: alert vs. drowsy, $P < .001$; alert vs. morphine, $P < .01$; drowsy vs. morphine, $P < .05$; and morphine vs. nalorphine, $P < .01$. It is concluded that morphine elevates the tooth pulp threshold more than does drowsiness alone.
(Supported in part by grant MY 2653 C1, USPHS).

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Pharmacology

ROLE OF CENTRAL NERVOUS SYSTEM CATECHOLAMINES IN MORPHINE HYPERGLYCEMIA. H. A. Campos* (intr. by F. E. Shideman). Dept. of Pharmacology and Toxicology, Univ. of Wisconsin, Madison.

Morphine hyperglycemia is believed to result from stimulation of central sympathetic centers and a subsequent discharge of adrenomedullary amines. Cats received reserpine (0.5-7.0 mg/kg, i.p.) 48 hours prior to producing a hyperglycemia with morphine (10 mg/kg, s.c.). Concentrations of blood sugar were followed for a period of 5 hours and the levels of catecholamines in the hypothalamus, the remainder of the brain stem and the adrenals were determined 24 hours after the injection of morphine. Similar data were obtained from cats which received only reserpine. In this group of animals tests for functional activity of the peripheral sympathetic nervous system were performed. All doses of reserpine produced approximately the same degree (70-90%) of depletion of catecholamines in the brain. The lowest dose of reserpine did not appear to have any significant effect on peripheral sympathetic activity and did not block the hyperglycemic response to morphine whereas the highest dose did block this response and reduced peripheral sympathetic function. These findings indicate that the hyperglycemic response to morphine occurs in the presence of a markedly reduced content of catecholamines in the central nervous system but is dependent on a relatively normal level of activity of the peripheral sympathetic nervous system.

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Pharmacology

THE ACTIVE FORM OF THE NARCOTIC ANALGESIC MOLECULE. Sherwood H. Biers and George W. Stevenson (intr. by D.J. Jenden) Dept. of Pharmacology, Univ. of Calif. Medical School, Los Angeles, Calif.

As a part of a study to determine whether the cationic or the uncharged form of various drug bases is the active form, a series of ω-cyanoalkyl narcotic analgesics has been synthesized, their ionization constants determined, and their analgesic activities tested in mice. The following N-normeperidine derivatives were prepared: -acetonitrile, -propionitrile, -butyronitrile, -valeronitrile, and -capronitrile. Their pK_a's were: 3.1, 6.2, 7.8, 8.5, and 9.0, respectively, while the pK_a of meperidine is 8.7. Using a tail pressure method, the -acetonitrile, -propionitrile, and -butyronitrile produced no analgesia whereas the -valeronitrile, and -capronitrile produced analgesia. The -acetonitrile and -propionitrile also showed some CNS stimulation. Since the majority of molecules of the first 2 members of the series are uncharged at pH 7.4, whereas the majority of molecules of the last 2 members, as well as meperidine, exist as the cation at pH 7.4, support is given to the hypothesis that the cation is the active form. (Supported by a grant from the U.S. Public Health Service.)

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Pharmacology

A METHOD FOR EVALUATING WEAK ANALGETICS IN PATIENTS WITH CHRONIC PAIN. C. H. Farr* and R. W. Payne, Depts. of Pharmacology and Med., Univ. of Okla. Sch. of Med. Okla. City, Okla.

A DOUBLE-BLIND METHOD FOR EVALUATING DRUGS ADAPTABLE TO AN OUT-PATIENT POPULATION HAS BEEN DEVELOPED. AFTER INITIAL ORIENTATION, CONTACT WITH PATIENTS IS MAINTAINED BY MAIL IN AN EFFORT TO MINIMIZE BIAS. DAILY EVALUATION CARDS FROM WHICH CAN BE DERIVED INFORMATION AS TO DEGREE OF PAIN SUFFERED DURING 4 PERIODS OF THE DAY ARE RETURNED BY EACH PATIENT PRIOR TO SENDING THE NEXT MEDICATION. A 6-MEMBERED DOUBLE-BLIND CROSS-OVER STUDY OF WEAK ANALGETIC AGENTS, USING TREATMENT PERIODS OF 1 WEEK, HAS BEEN COMPLETED BY THIS METHOD. THE STUDY WAS COMPRISED OF 32 ARTHRITIC PATIENTS WHOSE DISEASE WAS ACCOMPANIED BY CHRONIC PAIN. PATIENT COOPERATION WAS CONSIDERED EXCELLENT, WITH APPROXIMATELY 90% OF THE PATIENTS COMPLETING THE ENTIRE SERIES OF DRUGS. ASPIRIN, 650 MG. PROVED TO BE SIGNIFICANTLY MORE EFFECTIVE THAN PLACEBO FOR THE RELIEF OF MORNING PAIN WHILE ASPIRIN 650 MG. PLUS CODEINE, 32 MG. PROVED TO BE MORE EFFECTIVE THAN PLACEBO FOR THE RELIEF OF BOTH MORNING AND NIGHT PAIN IN THESE PATIENTS. NEITHER OF THESE DRUGS HOWEVER PERFORMED SIGNIFICANTLY BETTER THAN PLACEBO OVER THE TOTAL TREATMENT PERIOD OF ONE WEEK.

20

Pharmacology

FURTHER EVIDENCE FOR THE INCREASED EFFECTIVENESS OF PLACEBOS AND "ACTIVE" DRUGS WITH INCREASED STRESS. Henry K. Beecher. Mass. Gen. Hosp., Boston, Mass.

It will be shown that placebos are more than ten times as effective in relieving pain of pathological origin as they are in relieving experimentally contrived pain. This is construed as further evidence that placebos are more effective when stress is great than they are when it is less. A similar situation holds with morphine. Certain drugs are effective in relieving visceral sensations only if an essential psychological state is present.

177. NEUROPHARMACOLOGY–Anesthetics

1

Physiology

CHLORPROMAZINE PREMEDICATION WITH PENTOBARBITAL ANESTHESIA IN THE TURTLE. R. Young,* H. M. Kaplan, and M. R. Kaplan.* Southern Illinois Univ., Carbondale, Ill.

Adult temperature-adapted turtles of the species Pseudemys scripta were readily brought into deep anesthesia with an intramuscular injection of 10 mg/kg of chlorpromazine hydrochloride S.K.F. (Thorazine) followed after 10 min. by an intracardiac injection of 10 mg/kg of sodium pentobarbital in aqueous solution. The range of external temperature was 20°-23°C. The total time needed to induce deep (surgical) anesthesia averaged approximately 15 min. from the start of injection. The average duration of anesthesia at all depths was approximately 4 hr., and that of deep anesthesia proper was approximately 3 hr. Both the heart rate and the rectal temperature increased in the first 90 min. after the injection of chlorpromazine and pentobarbital. The rise in temperature was slight. A corneal reflex was maintained during anesthesia. The diameter of the pupil was always slightly decreased. Muscle tone was lost in 87% of the animals at 30 min. and in 94% at 60 min. after the injection of both drugs. Occasional localized gross movements were present in variable degrees in 29% of the animals at 30 min. and in 17% of the animals at 60 min. following the administration of both drugs. A shorter induction time is obtainable with pentobarbital by chlorpromazine premedication.

2

Biochemistry

COMPARATIVE HYDROLYSIS OF CERTAIN DENTAL LOCAL ANESTHETICS BY HUMAN SERUM AND PLASMA. Charles E. Becker (intr. by J. Roy Doty.) American Dental Association, Chicago, Illinois.

Continuation of work (C. E. Becker, Fed. Proc. 18: 188, 1959) has shown that 180 micromoles of acetylcholine at pH 8.0, 38.6 micromoles of metabutethamine, 19.5 micromoles of meprylcaine, 5.9 micromoles of piperocaine, 1.3 micromoles of procaine, and 0.7 micromoles of butethamine at pH 7.4 were hydrolyzed per ml of serum per hr at 37°C. Corresponding values for plasma from the same individual were 68, 82, 93, 98, 70, and 100 percent, respectively, of the above serum values. In paper electropherograms prepared with serum and the TEB buffer of Aronsson and Gronwall at pH 8.9 (Scand. J. Clin. Lab. Invest. 9: 338, 1957) or barbital buffer at pH 8.6, eluates from the alpha$_2$- and beta-globulin zones hydrolyzed each of the six substrates. In addition with the TEB buffer but not with barbital, eluates from a zone which corresponded to albumin and alpha$_1$-globulin hydrolyzed each of the substrates except acetylcholine and procaine. These results are compatible with the presence of more than one pseudocholinesterase in human blood. (Supported in part by PHS Grant D-533.)

3

Pharmacology

SERIES OF FLUORINATED ETHERS. Alan Van Poznak* and Joseph F. Artusio, Jr. New York Hosp. and Cornell Univ. Med. College, New York City.

In a search for a nonflammable ether producing the favorable physiological conditions found with diethyl ether, a series of halogenated methyl ethyl ethers was administered to nonfasting mongrel dogs. Oxygen was used as the diluent in all cases. Heidbrink machines with Baralyme as the CO_2 absorbent were used. A total of 19 anesthesias, using 7 different agents, was given. Complete substitution of hydrogens on the methyl portion of the ether resulted in convulsants exemplified by CF_2ClOCF_2CHFCl and $CFCl_2OCF_2CHF_2$. Partial substitution on the methyl portion resulted in less but still definite irritative action as shown by CH_2ClOCF_2 CHClF. Satisfactory immediate anesthesia with delayed deaths was produced by $CH_2ClOCF_2CF_2H$. CH_3OCF_2CHFCl was a satisfactory but flammable anesthetic. The best results were obtained with the nonflammable agents $CH_3OCF_2CHCl_2$ and $CH_3OCF_2CFClBr$. Of these, the former is more light stable and of lower molecular weight. Arrhythmias produced by intravenous epinephrine 5 gamma/Kg. in the conscious dog were lessened after anesthesia with $CH_3OCF_2CHCl_2$. Ventilation was full and even. Cardiovascular depression was minimal. There was no vomiting; salivation was minimal. Cautious trial in humans seemed warranted.

4

Pharmacology

CLINICAL EVALUATION OF METHOXYFLURANE IN MAN. J.F. Artusio, Jr., A. Van Poznak*, R. Hunt*, F. Tiers*, and M. Alexander*. New York Hosp. and Cornell Univ. Med. College, New York, N.Y.

Previous studies with 1, 1, dichloro 2, 2, difluoro ethyl methyl ether (Methoxyflurane) in dog warranted a clinical trial in man. Methoxyflurane boils at 104.8°C. at 1A. Explosion limit in air and O_2 at 20°C. is zero. Solubility in water is 0.095/100 gm. H_2O and is miscible in olive oil in all proportions. Saturated vapor pressure is 25 mm. Hg. at 20°C. This agent was given by open drop, semi-closed and closed techniques. Induction time in 9 minutes without excitement. Recovery was without delirium or hypotension. Nausea and vomiting were minimal. Respiration was only slightly depressed during surgical anesthesia and circulatory hypotension was a sign of overdose. Compatibility with d-tubocurarine and succinylcholine has been demonstrated. A wide variety of surgical procedures have been accomplished during administration of this agent in 100 patients. EEG was not a precise indicator of depth of anesthesia. ECG indicated a stable, normal sinus rhythm. Eyeballs are fixed and pupils remain small throughout surgical anesthesia. Peripheral muscle relaxation was marked at minimal levels of central nervous system depression. Liver function as measured by BSP retention indicated hepato-toxicity of the same magnitude as found by Fairlie (N.Eng.J.Med. 244: 617, 1951) for di-ethyl ether.

5

Pharmacology

EFFECT OF HALOTHANE ON GASTROINTESTINAL MOTILITY. F. N. Marshall*, C. B. Pittinger and J. P. Long. Department of Pharmacology and Division of Anesthesiology, Department of Surgery, College of Medicine, State University of Iowa, Iowa City.

Studies were conducted in dogs to investigate the effect of halothane (2-bromo-2-chloro-1,1,1-trifluoroethane) on gastrointestinal motility. Jejunal activity was recorded from a balloon placed in a Thiry-Vella loop. Some animals had a portion of cecum exteriorized to facilitate insertion of a balloon directly into the colon. Gastric motility was transmitted from a balloon passed through the esophagus. By these procedures, simultaneous recordings from stomach, jejunum and colon were feasible. Increasing concentrations of halothane exerted a progressive depressant effect at these sites. Complete abolition of motility occurred before severe respiratory depression. Depression and recovery at the three sites occurred almost simultaneously. The stimulant effect of morphine on the jejunum is antagonized by halothane.

XUM

6 *Pharmacology*

BEHAVIOR OF METHOHEXITAL IN MAN. Lester C. Mark, Leonard Brand*, Peter Dayton*, Patricia Blaber* and E. M. Papper. Department of Anesthesiology, Columbia University and New York University Research Service, Goldwater Memorial Hospital, New York City And Laboratory of Chemical Pharmacology, National Heart Institute, Bethesda, Maryland.

The barbiturate methohexital (Brevital) has recently been introduced as an ultrashort-acting intravenous anesthetic. Studies in human subjects showed that onset of sleep and of characteristic changes in the electroencephalogram occurred promptly after administration. Despite relatively large doses (1.4 to 1.8 grams) spontaneous respiration was resumed within 10 to 35 minutes after end of infusion. Return to complete awareness although delayed was somewhat more prompt than with thiopental. Plasma decay curves showed that after diffusion equilibrium was established the rate of disappearance from plasma was 15 to 19 per cent per hour. Analysis of samples of fat tissue obtained at operation indicated entry of drug into fat. Despite the disadvantages of hiccups, twitching and shivering observed during its administration the drug warrants further study. (Supported in part by Contract No. DA-49-007-MD-629, Office of the Surgeon General, Department of the Army and in part by Grant No. B-1288(C1,2), National Institute of Neurological Diseases and Blindness, Public Health Service.)

7 *Pharmacology*

EFFECTS OF HALOTHANE (FLUOTHANE) ANESTHESIA ON VENTRICULAR CONTRACTILE FORCE IN THE HUMAN AND DOG. W. B. Thrower*, T. D. Darby, E. E. Aldinger* and J. H. Sprouse*. Depts. of Pharmacology, Surgery and Anesthesia. Medical College of South Carolina, Charleston.

Ventricular contractile force (VCF), electroencephalogram (EEG), electrocardiogram (ECG) and arterial pressure (BP) changes were recorded during thoracotomy in five patients. With anesthesia which produced predominantly low frequency EEG waves (1 cps - 100 microvolts) VCF was depressed to 20 percent of control values. BP decreased by 25 to 30 mm of Hg mean pressure. ECG recordings showed bradycardia with sinus rhythm. Lower vapor concentrations of halothane (0.5% to 1.0%) produced only minimal changes in VCF (80 to 85% of control values) the ECG at this time showed frequencies of 15 to 30 cps - 40 microvolts. Studies in 10 dogs have shown comparable depression of VCF at comparable anesthesia levels. Extra corporeal circulation studies in dogs have shown that the myocardial depression is not secondary to a decrease in coronary flow due to hypotension. Preliminary results support the view that an element of sympathetic depression is involved in the hypotension and decrease in VCF produced by halothane. (Supported by grants from National Heart Institute, S. C. Heart Assoc. and Am. Heart Assoc.)

8 *Pharmacology*

EFFECT OF ANESTHESIA ON PREVIOUSLY IRRADIATED SMALL ANIMALS. T. M. Young*, J. S. Mezistrano*, S. A. A. Carson*, and L. E. Morris, Division of Anesthesiology, University of Washington School of Medicine, Seattle, Washington.

Zauder and Orkin (Fed. Proc. March 1959, 18:1 1829) showed in mice that post radiation mortality was significantly increased after anesthesia, the magnitude of the effect varying with both the dose of radiation and the anesthetic drug. Apart from this paper there is a startling lack of information on this subject. Yet the obvious implications of atomic disaster necessitate a full understanding of the relationship of anesthesia to irradiation. Mice were irradiated in single doses varying from 200 to 1100 R. The LD 50 of the unanesthetized controls was 650R. Inhalation anesthetics were administered at various intervals thereafter. Subsequent changes in weight and survival rates were uniformly modified unfavorably in the group which received anesthesia immediately after irradiation. In these circumstances Fluothane appeared to be least toxic of the drugs used. Diethyl ether, Fluoromar, and chloroform exhibited increasing adverse effect in that order.

9 *Pharmacology*

ELECTROCARDIOGRAPHIC STUDIES WITH SYNTOCINON AND PITOCIN DURING INHALATION AND REGIONAL ANESTHESIA. E. T. Capps and John W. Berry*. Div. of Anesthesiology, Univ. of Washington School of Med. Seattle, Wn. and Univ. of Texas Southwestern Med. School, Dallas, Texas.

Electrocardiograms were obtained from obstetrical patients at the time of administration of the oxytocics Pitocin and Syntocinon following delivery. Control tracings were obtained with the patient in the delivery room prior to delivery, and records were made at intervals of one minute for ten to twenty minutes after the oxytocics had been administered intramuscularly or intravenously following delivery of the infant. Data was obtained during the use of the inhalation agents nitrous oxide, cyclopropane and trichlorethylene and the regional agents tetracaine, used as a saddle block, and lidocaine, used as a nerve block or by infiltration. The electrocardiograms obtained following administration of the oxytocics were compared with control tracings for changes in cardiac rate and rhythm, P-R interval, height of T wave and displacement of S-T segment.

10 *Pharmacology*

CENTRAL EFFECTS OF LOCAL ANESTHETICS. H.W. Elliott, G.C. Quilici* and C. Elison*. Univ. of Cal. Med. Center, San Francisco 22,

The toxicity of local anesthetics on the CNS is manifested by stimulant and depressant effects. The former seem confined to the cortex but the latter may affect the entire brain under the proper conditions as shown by the successful use of lidocaine as an anticonvulsant. Our studies in dogs indicate that local anesthetics given IV deepen barbiturate anesthesia as judged by respiratory depression and EEG changes. With lidocaine, procaine, tetracaine and chloroprocaine this occurred without cardiovascular effects but when cocaine was used, the central effects were accompanied or preceded by cardiac arrythmias and a profound fall in blood pressure. Awake, restrained dogs did not sleep during continuous IV infusions of lidocaine but appeared sedated unless the rate of infusion was sufficient to cause convulsions. EEG activity in unanesthetized curarized dogs given similar infusions resembled that seen in barbiturate anesthesia except that increasing the dose produced a convulsive EEG pattern rather than an isoelectric EEG. When lidocaine or procaine were applied locally to the cerebral cortex or injected into the left lateral ventricle of unanesthetized curarized dogs EEG patterns of central depression were observed and in the latter case, cardiac toxicity as well. Convulsive EEG patterns were produced in these animals when the same drugs were given IV. (Aided by grant from The NIH.)

11 *Pharmacology*

CYCLOPROPANE-EPINEPHRINE SYNERGISM ON NICTITATING MEMBRANE. J. S. Gravenstein, E.T. Sherman and T.W. Andersen. (intr. by T.H. Maren) Division of Anesthesia, College of Medicine, University of Florida, Gainesville, Florida.

Reports on cyclopropane (Cy) "sensitization" of muscle to the effects of epinephrine (E) are limited to the heart. However, this synergism must be expected to occur in other organs responding to E. The nictitating membrane (NM) of the spinal cat was tested. Preganglionic sympathetic fibers supplying the NM were cut, the adrenals were removed, and 1 mg/kg atropine given. Cy in concentrations of 10, 20, 30, and 40% in O_2 administered for 5 min each did not cause contractions of the NM. Continuous infusion of E in concentrations of 0.3, 1, 3, and 10 μg/kg/min was tested. The lowest concentration was without effect, the higher ones caused sustained contraction of the NM. When Cy 10, 20, 30, 40% was administered during the continuous E infusion, it regularly and markedly enhanced the E contractions of the NM. Single doses of E were given after Cy had been eliminated (but while the E infusion was continued) until the contractions of the NM seen with Cy were reproduced. Thus, it was found that approximately 1, 10, and 69 μg/kg E were required to reproduce the contractions due to 40% Cy during continuous infusions of 1, 3, and 10 μg/kg/min E respectively. Cy is believed to be a non-specific synergist enhancing contractions of both smooth and striated muscles.

179. NEUROPHARMACOLOGY–Anticholinergic Agents

Pharmacology

PARASYMPATHOLYTIC ACTIVITY OF TERTIARY AMINES AND QUATERNARY AMMONIUM COMPOUNDS. F. P. Luduena. Sterling-Winthrop Res. Inst., Rensselaer, N. Y.

It is known that parasympatholytic quaternary ammonium compounds(Q) are more active than the corresponding tertiary amines(T). In order to investigate the nature of this difference the activity of the following compounds was determined on the rat colon in vitro (antiacetylcholine effect) and by subc. inj. in mice (mydriatic effect): atropine sulf., atropine methyl nitrate, d-1 and l-trihexyphenidyl and their methiodides, 2-diethylaminoethyl α-cyclohexyl-α-(1-hydroxycyclopentyl) acetate(I) and three ring homologues (II, III, IV) and the methobromide salts of I, II, III and IV. In all cases (Q) were more active than (T) but the differences were much greater in vivo (Q/T molar act. ratios: 4.8 to 74.) than on the rat colon (mol. act. ratios: 1.1 to 3.1). In the homologous series the Q/T mydriatic ratio increased with mol. weight. It is concluded that at the molecular level the Q/T activity ratio is relatively low and that the high ratios obtained in vivo are due mainly to differences in the (Q) and (T) distribution in the body.

Pharmacology

ANTAGONISTIC EFFECTS OF METHACHOLINE ON CENTRAL AND PERIPHERAL ACTIONS OF HYOSCINE IN MAN. A. M. Ostfeld and A. Y. Coombs (intr. by K. R. Unna). Depts. of Preventive Medicine and Pharmacology, Univ. of Ill. Coll. of Med., Chicago, Ill.

Four groups of five normal volunteers were employed in a double blind 2 x 2 factorial design. All groups were given first either a high (.8 mg I.M.) or low (.2 mg I.M.) dose of hyoscine followed in one hour by a high (50 mg I.M.) or low dose (5 mg I.M.) of methacholine. Physiological and psychological measures were taken before, between, and after drugs. Hyoscine significantly increased pupil diameter and decreased salivation. Methacholine significantly reversed the salivary but not the pupil effects of hyoscine. Hyoscine significantly decreased ratings of behavioral alertness, speed of writing "A"'s, capacity to recall numbers, performance on the Stroop test, and the Energetic scale score of the Clyde Mood Scale. Methacholine significantly reversed the effects of hyoscine on behavioral ratings, speed of writing "A"'s, and capacity to recall numbers but had no effect on the hyoscine-induced changes in the Stroop test or Clyde Mood Scale. (Supported by Grant MY 2302 from the National Institutes of Health)

180. NEUROPHARMACOLOGY–Cholinesterase Inhibitors

Physiology

EFFECT OF 5-HYDROXYTRYPTAMINE ON CHOLINESTERASE ACTIVITY. M. H. Aprison. Institute of Psychiatric Research, Indiana University Medical Center, Indianapolis, Indiana

A number of facts suggest that the acetylcholine-cholinesterase and 5-hydroxytryptamine-monoamineoxidase systems might interact. The effect of serotonin on brain cholinesterase (ChE) from rabbit caudate nuclei homogenates was determined. An automatic titrator was used to assay the ChE activity at constant pH(7.8), temperature (37°C) and substrate concentration ($3x10^{-3}$M). Final serotonin concentration of $9x10^{-4}$M, $2.4x10^{-3}$M, $4.9x10^{-3}$M or $9x10^{-3}$M resulted in inhibition of 29, 43, 63, and 100 percent respectively. 5-hydroxytryptophan (5-HTP) also inhibited ChE activity. Serotonin inhibition was found to be neither all competitive nor all non-competitive but intermediate, that is, the Lineweaver-Burk plots yielded curved lines. Similar results were obtained with purified erythrocyte ChE. Since marked inhibition occurs only at fairly high concentrations of serotonin its action at normal physiological levels might be to compete with ACh for the ACh-receptor protein whereas at elevated levels, such as after administration of large doses of 5-HTP, it inhibits ChE. Thus the sedation effect seen after moderate doses of 5-HTP might be the result of the block of normal ACh action whereas the stimulatory effect seen after large doses of 5-HTP might be due to the increased accumulation of ACh after ChE inhibition.

Biochemistry

INHIBITION OF BRAIN AND PLASMA CHOLINESTERASES AND PARALYTIC EFFECTS OF ORGANIC PHOSPHATE COMPOUNDS. Robert F. Witter and Thomas B. Gaines† Toxicology Section, Technical Development Laboratories, Technology Branch, Public Health Service, U. S. Department of Health, Education, and Welfare, Savannah, Ga.

A study was made of the relationship between the ability of certain organic phosphorus compounds to produce a syndrome of "muscle weakness" and the in vivo lowering of plasma and brain cholinesterases after subcutaneous administration of the compound to chickens. At the levels tested, all compounds studied caused a marked drop in the brain true and/or pseudocholinesterase. The brain enzymes recovered in activity to within 70 percent of normal within 10 days in the case of non-paralytic compounds, DDVP, and Dipterex, but remained markedly inhibited for from 10 to 21 days with the paralytic compounds, TOCP, malathion, EPN, ronnel, methyl parathion, and Trithion. TOCP inhibited the pseudocholinesterase more effectively than the true cholinesterase of brain; the reverse was true with the other paralytic compounds. No generalization could be made about the effects on the plasma enzyme since these depended solely on the compound under investigation.

Pharmacology

EFFECTS OF COMBINATIONS OF SYSTOX AND METHYL PARATHION IN THE DOG. Virginia L. Zaratzian,* Henry N. Fuyat,* and O. G. Fitzhugh, Division of Pharmacology, Food and Drug Administration, U.S. Dept. of Health, Education, and Welfare, Washington, D.C.

It has been reported that the daily concomitant administration of official tolerance levels of methyl parathion (1 ppm) and Systox (0.75 ppm) to humans resulted in hypoprothrombinemia, eosinophilia and lymphocytosis (Moeller & Rider, Fed. Proc. 18:424, 1959). These effects were not observed when the same organic phosphates were given alone. Studies were initiated to determine the effects of this combination on prothrombin time and esterase activity of plasma and red cells in the dog. Dosage levels of 0, 0.025, 0.125, or 0.250 mg/kg of methyl parathion and 0.019, 0.05, or 0.075 mg/kg of Systox, and combinations of the two low intermediate, and high levels were administered six days per week in capsule form for twelve weeks. Methyl parathion had little effect on plasma or red cell esterase activity. Systox had some inhibitory effect on plasma but not on red cell esterase activity. No potentiation occurred at these levels. There were no effects on prothrombin time.

Pharmacology

PHARMACOLOGIC STUDIES ON A NEW SERIES OF ANTICHOLINESTERASES. R. H. Adamson*, D. Boatman*, and J. P. Long. Dept. of Pharmacology, College of Medicine, State University of Iowa, Iowa City.

Some of the pharmacologic properties of a series of bis quaternaries have been evaluated. The compounds are disubstituted 1,5-diaminonaphthylenes where substitutions on the amino group are 2-methylpyridyl, N-acetyl (I), 2,3-dimethylpyridyl, N-acetyl (II), 2,6-dimethylpyridyl, N-acetyl (III), pyridyl, N-acetyl (IV), 4-methylpyridyl, N-acetyl (V), 3-methylpyridyl, N-acetyl (VI), 1,3-dimethylpyridyl, N-acetyl (VII) and dimethylaminoethanol, N-acetyl (VIII). In vitro anticholinesterase activity was determined by standard manometric techniques (Umbreit et al., 1949). The substrate employed was 0.02 M acetyl-β-methylcholine and the enzyme was bovine erythrocyte cholinesterase (Winthrop). The approximate molar I50 values found were : I, 1.2×10^{-7}; II, 9.0×10^{-6}; III, 5.0×10^{-6}; V, 5.1×10^{-6}; VI, 5.5×10^{-6}. The ability of the compounds to antagonize the actions of d-tubocurare was studied in vivo on the severed, rabbit sciatic nerve-gastrocnemius muscle preparation and compared with similar intravenous doses of neostigmine. Compounds II, IV and V compared favorably with the actions of neostigmine in this preparation. I, III, VI and VII were somewhat less active and VIII was virtually inactive.

XUM

5

Pharmacology

PHARMACOLOGICAL ACTIVITY OF GALANTHAMINE AND RELATED COMPOUNDS. Richard L. Irwin (intr. by F. J. Macri) National Institute of Neurological Diseases and Blindness, Bethesda, Maryland.

The alkaloids galanthamine and lycoramine have been isolated recently from a number of Amaryllidaceae plants. Galanthamine HBr has been reported to be useful in the treatment of myasthenia gravis. (Sedova K.1956 Notes on New Medicinal Agents, p.14-16 Medgiz, Moscow) Thus the potentiation of muscle contraction and the inhibition of muscle cholinesterase have been studied. Galanthamine methiodide, lycoramine methiodide and deoxydemethyl lycoramine methiodide produced marked potentiation of muscle twitches at dose levels below 0.5 mg/Kg. Deoxydemethyl lycoramine methiodide was the most potent compound examined. Galanthamine HBr was active at 1-2 mg/Kg. Lycoramine acetate methiodide, neopine HCl and neopine methiodide failed to potentiate muscle contraction at 5.0 mg/Kg. Galanthamine methiodide is more effective in inhibiting muscle cholinesterase than edrophonium but less potent than neostigmin or physostigmine. Deoxydemethyl lycoramine methiodide has inhibitory efficacy equal to that of neostigmin or physostigmine.

6

Pharmacology

TRANSITIONS BETWEEN ACTIVATION AND INHIBITION OF PSEUDOCHOLINESTERASE. W. Kalow. Dept. of Pharmacology, Univ. of Toronto, Toronto 5, Canada.

It has been known for sometime that several alkaloids and quaternary ammonium compounds are capable of both inhibiting and activating cholinesterase. Using tetramethylammonium (TMA) systematically as a model inhibitor, some conditions which cause a transition from esterase inhibition to activation have been studied in a pH-stat. Sources of enzyme were several partly purified preparations of normal and atypical cholinesterase of human serum. Acid pH and low TMA concentrations tended to cause enzyme activation. The hydrolysis of benzoylcholine was more easily activated than that of acetylcholine; high substrate concentration favoured activation. Normal and atypical cholinesterase showed an identical pattern of responses but quantitative differences. The experiments suggest the existence of more than one binding site per enzyme molecule. The data seem to reflect pharmacological differences between TMA and tetraethylammonium (TEA).

7

Pharmacology

BLOCKING AND FACILITATORY EFFECTS OF THIOPHOSPHONATE COMBINATIONS. Alexander G. Karczmar and K. Blachut*. Dept. of Pharmacology and Therapeutics, Grad. School and Stritch School of Med. of Loyola University, Chicago 12, Ill.

Potentiation rather than additive synergism of toxicity arises upon combined administration of thiophosphonate anticholinesterases (Frawley et al., J. Pharmacol. and Exper. Therap., 121: 96, 1957; Karczmar et al., Fed. Proc., 17: 232, 1958 and 18: 409, 1959). Conspicuous potentiation following phosphonate combinations arises also at neuromyal junction and at the central nervous system; it may be slight at other neuroeffectors (Karczmar, o.c.). Toxicity and neuroeffector phenomena arise when subliminal doses of EPN are followed after 30-60 minutes by Malathion; no toxicity arises when this sequence is reversed. Besides facilitatory actions, thiophosphonates have also blocking actions; at salivary neuroeffectors phosphonate combinations produce blockade additively. At neuromyal junction and at salivary neuroeffectors oxime "reactivators" such as 2-PAM and TMB-4 produce blockade when given following subliminal doses of thiophosphonates; on the other hand, they reverse certain apparently anticholinesterase effects of thiophosphonate combinations. (Supported in part by PHS Grant B-1368.)

8

Pharmacology

ANTICHOLINESTERASE ACTIONS OF THIOPHOSPHONATE COMPOUNDS (EPN AND MALATHION) AND THEIR COMBINATIONS. O. Awad* and Alexander G. Karczmar, Dept. of Pharmacology and Therapeutics, Grad. School and Stritch School of Med. of Loyola University, Chicago 12, Ill.

EPN and Malathion were found to inhibit in vivo brain acetylcholinesterase of dogs (Awad, Fidlon and Karczmar, Fed. Proc., 18: 364, 1959). It was found at present that potentiation of this effect arises upon administration of EPN-Malathion combinations. However, presence or absence of the potentiation depends on the sequence of administration. Potentiation did not occur within 30 minutes of administration of thiophosphonate combinations to hepatectomised dogs. In vitro, combinations of EPN and Malathion inhibit weakly and act only additively. Both EPN and Malathion form irreversible complexes with acetylcholinesterase as shown in dilution and dialysis experiments. This, the dependance of potentiation upon sequence of administration and pharmacologic data presented elsewhere at this Meeting (cf. Karczmar and Blachut, this issue) seem to militate against possibility of potentiation being due to one thiophosphonate protecting the other from destruction by esterases. (Supported in part by PHS Grant B-1368.)

9

Pharmacology

PROTECTION AGAINST ALKYLPHOSPHATE INTOXICATION BY INTRAVENTRICULAR INJECTION OF PYRIDINE-2-ALDOXIME METHIODIDE. Howard Tong* and James L. Way. Dept. of Pharmacology and Toxicology, Univ. of Wis. Med. Sch., Madison.

In an attempt to evaluate the central action of pyridine-2-aldoxime methiodide (PAM) in protecting against alkylphosphate intoxication, PAM was injected either intraperitoneally (90 mg/kg) or intraventricularly (5 mg/kg) according to the method of Haley and McCormick (Brit. J. of Pharmacol. 12: 12, 1957) and intraperitoneally (55 mg/kg) in mice. The LD_{50} values of tetraethylpyrophosphate in groups of mice premedicated with PAM alone or in combination with atropine sulfate (10 mg/kg) were compared. The statistical data were analyzed by the method of Litchfield and Wilcoxon (J. Pharmacol. & Exper. Therap. 96: 99, 1949). The results indicated that the combined intraventricular and intraperitoneal administration of PAM was most effective in antagonizing tetraethylpyrophosphate intoxication and this effect was greatly potentiated when tested in combination with atropine sulfate.

10

Pharmacology

METABOLISM OF PYRIDINE-2-ALDOXIME C^{14} METHIODIDE BY LIVER PERFUSION. James L. Way, Howard Tong* and Richard Rabideau*. Dept. of Pharmacology and Toxicology, Univ. of Wisconsin Med. School, Madison, Wisconsin.

Carbon-labeled pyridine-2-aldoxime methiodide (2-PAM) was synthesized by the interaction of C^{14} methyl iodide and pyridine-2-aldoxime according to the method of Ginsberg and Wilson (J. Am. Chem. Soc. 79: 481, 1957) in a yield of 95 per cent based on methyl iodide. The metabolism of radioactive 2-PAM was studied by perfusion of isolated rat livers. Ultraviolet spectrophotometry and the method of Csaky (Acta. Chem. Scand. 2: 450, 1948) were employed to measure the rate of metabolism. The half-life of 140 micromoles of 2-PAM was about six hours. Approximately 5 per cent of the radioactivity was excreted in the bile. Chromatographic fractionation of the alcohol-soluble portion of the perfusate on Dowex 50-sodium columns with varying concentrations of sodium chloride revealed three radioactive fractions. The tubes containing each of the radioactive fractions were pooled separately, adsorbed on Darco S-51 charcoal columns, eluted with 50 per cent ethanol and dried in vacuo. One of the radioactive fractions liberated cyanide when interacted with 0.1 N KOH for one minute and possessed ultraviolet spectral properties similar to those of 2-cyanopyridine methiodide.

1 *Pharmacology*

ACETYLCHOLINESTERASE INHIBITION BY TETRAMETHYLAMMONIUM AND EXCESS SUBSTRATE. Arthur K. Cho* and Donald J. Jenden. UCLA Med. Center, Los Angeles, Calif.

Rates of acetylcholine (ACh) hydrolysis by acetylcholinesterase were measured by constant pH titration at 30° C with an accuracy of ≤ 1% over a substrate concentration range of 2.8×10^{-4} M to 3.8×10^{-2} M, and in the presence of tetramethylammonium chloride (TMA) concentrations from 1.3×10^{-3} M to 1.9×10^{-1} M. Acetylcholinesterase was prepared from Electrophorus electric organ by fractional ammonium sulfate precipitation, and was capable of hydrolysing 80 μ mol ACh per mg protein per sec. Mg^{++} did not accelerate the rate of hydrolysis, and was omitted from the reaction mixture. The data are quantitatively consistent over the whole concentration ranges with the existence of two anionic sites, for which substrate and inhibitor compete. If both sites are occupied by ACh, no hydrolysis occurs. If one site is occupied by TMA, the other is still capable of binding substrate, and hydrolysis may result; the rate constant for ES → E + Products is however reduced by a factor of 3.0, and the Michaelis constant is increased by a factor of 15.9. The dissociation constants for the two anionic sites with TMA are 2.8×10^{-4} and 7.6×10^{-2} mol. l^{-1}; the Michaelis constant is 3.29×10^{-4} mol. l^{-1}, and the dissociation constant for substrate at the second site is 3.73×10^{-2} mol. l^{-1}. (Supported by a grant from the United States Public Health Service.)

181. NEUROPHARMACOLOGY–
Monamine Oxidase Inhibitors

1 *Physiology*

THE ALTERATION OF EPINEPHRINE METABOLISM IN MAN. O. Resnick, M. Hagopian and H. Freeman (intr. by George P. Fulton). Worcester Foundation for Experimental Biology, Shrewsbury, Mass. and Medfield State Hospital, Medfield, Mass.

We have previously reported that Marsilid treatment in man inhibits the action of the monoamine oxidase (MAO) enzyme system on the metabolism of infused epinephrine (E), but does not influence the O-methyl transferase system (Science 127: 1116, 1958). In the present study, the influence of Marplan, 1,2,3,4-tetrahydrophthalazine.HCl (RO 5-0994), and Isoniazid on the metabolism of infused E was investigated. Treatment with 30-40 mg. per day of Marplan resulted in an inhibition of MAO activity equal to that following treatment with 100-200 mg. per day of Marsilid. 10-30 mg. per day of RO 5-0994 produced a good inhibition of MAO in one patient and a slight inhibition in two patients. Isoniazid which is known to have little or no influence on MAO activity produced no alteration of E metabolism. The clinical evaluation of these patients will be presented. (Supported by a grant from the U.S.P.H. Service MY-2936, Ford Foundation and Hoffmann-LaRoche, Inc.)

2 *Physiology*

EFFECT OF ISONIAZIDE AND IPRONIAZIDE ON THE PATHWAYS OF GLUCOSE UTILIZATION IN THE PERFUSED BRAIN. A. Geiger, V. Aguilar, G. Gombos, W. Scruggs, and G. Whitney (intr. by Frederic A. Gibbs.) Research Laboratories, Dept. of Psychiatry, Univ. of Illinois, Chicago.

In previous brain perfusion experiments with uniformly C^{14} labeled glucose in the perfusion blood, the C^{14} content of the CO_2 produced and of the free amino acids reached a maximum of 30% of the respective total carbon contents, after an initial exchange lasting 30-40 minutes and remained at this level, indicating a large steady influx of cold endogenous carbon into the metabolic pool. Isoniazide and iproniazide, both, changed this pattern by increasing steadily the proportion of C^{14} in the CO_2 produced until the C^{14} concentration reached 85 to 90% of the total in CO_2, indicating a blocking of CO_2 production from preformed endogenous sources. C^{14} incorporation into amino acids was similar to that into CO_2. The possible sites of the blockage and the biochemical mechanism of action of these drugs are discussed.

3 *Pharmacology*

OBSERVATIONS ON THE CARDIOVASCULAR EFFECTS OF TRANYLCYPROMINE. J. N. Spencer, M. Porter*, H. L. Froehlich*, and H. Wendel. Smith Kline & French Laboratories, Philadelphia, Pa.

The cardiovascular effects of the monoamine oxidase inhibitor (MAO-I), tranylcypromine ('Parnate'), was studied in dogs. In the anesthetized animal, the initial i.v. injection produced a sustained pressor response but subsequent injections resulted in a sustained hypotension. This occurred in the absence of any alteration in the response to the injection of epinephrine, norepinephrine or sympathetic ganglionic stimulation. The pressor response was blocked by 'Dibenzyline', indicating that it was peripheral in origin, while the depressor effect appeared to be associated with a central action. In view of the observed depressor action and the occurrence of hypotension in patients administered MAO-I, the effect of tranylcypromine and certain other MAO-I on the mean arterial blood pressure of unanesthetized dogs was investigated. The i.v. injection of tranylcypromine produced an immediate increase in blood pressure, followed by a slow decrease, hypotension developing in 6 to 24 hours. Oral doses of 2 mg/kg of tranylcypromine and certain other MAO-I produced a slight to moderate decrease in blood pressure. The decrease was evident within 24 hours, but that produced by tranylcypromine did not last more than 72 hours. This was a considerably shorter duration of hypotension than that obtained with certain other MAO-I.

4 *Pharmacology*

THE METABOLISM OF ISOCARBOXAZID (MARPLAN(T.M.)) IN THE RAT. Morton A. Schwartz (intr. by Lowell O. Randall.) Dept. of Pharmacology, Hoffmann-La Roche Inc., Nutley 10, New Jersey.

The monoamine oxidase (MAO) inhibitor, isocarboxazid (Marplan(T.M.)), chemically 1-benzyl-2-(5-methyl-3-isoxazolylcarbonyl)hydrazine, was synthesized with a C^{14} label in the benzyl moiety. A 1 mg/kg intraperitoneal dose of C^{14}-isocarboxazid was found to be rapidly absorbed and distributed in the rat with the radioactivity reaching maximum tissue levels within 7.5 minutes. The urinary radioactivity, which was virtually all accounted for as labeled hippurate by reverse isotope dilution analysis, amounted to 70% of the dose in 24 hours. Fecal excretion accounted for 7% of the dose. At a dose 40-fold higher (40 mg/kg), the urinary radioactivity amounted to 42.5% of the dose and labeled hippurate accounted for only 65% of the urinary radioactivity (28% of the dose); the fecal excretion of radioactivity amounted to 22% of the dose. Labeled benzylhydrazine was detected as a breakdown product in in vitro studies in which C^{14}-isocarboxazid was incubated with liver homogenates and slices in Krebs-Ringer phosphate buffer, pH 7.3. Apparently, benzylhydrazine is formed by hydrolytic cleavage of isocarboxazid, and is oxidized to benzoate which is excreted as hippurate.

5 *Pharmacology*

PHENELZINE - A NEUROPHARMACOLOGICAL STUDY. Jane Frances Emele*, John E. Shanaman* and Marshall R. Warren. Warner-Lambert Research Institute, Morris Plains, New Jersey.

The effect of phenelzine was studied on spinal cord reflexes of spinal and intact cats. Initial studies demonstrated that this substance predominantly inhibited monosynaptic (patellar reflex) transmission in the spinal cord of intact cats. Polysynaptic reflexes (flexor reflex) are affected less selectively. The clinical efficacy of phenelzine in endogenous depressions and the implication of the limbic system in emotion and behavior led to electroencephalographic studies. Unanesthetized cats with chronically implanted bipolar electrodes were used and correlations were made between the electroencephalographic effects and behavior. Single intravenous injections of phenelzine produced synchronous, low voltage, fast activity in the cortical areas concurrent with spike and wave complexes in the hippocampus. Data will be presented to show the effect of chronic administration of this drug on the EEG pattern.

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Pharmacology

IPRONIAZID REINFORCEMENT OF ANTICONVULSANTS. Helen C. Y. Yen, Arthur J. Silverman and Anita Salvatore (intr. by T. O. King.) Division of Pharmacology, Ortho Research Foundation, Raritan, N. J.

Recent studies by Prockop et al. (Experientia 15, 145, 1959) and Chow and Hendley (Fed. Proc. 18, 376, 1959) have demonstrated that monoamine oxidase inhibitors protect rats against maximal electroshock seizureand produce a rise in electroshock threshold in mice, respectively. We utilized this property of iproniazid to examine certain aspects of its effect upon anticonvulsants. This investigation was initiated by the finding that iproniazid at a dose of 25 mg./kg., orally in mice, is capable of lowering the oral ED_{50} (mg./kg.) against MES of anticonvulsants as follows: trimethadione 540 to 350, phenacemide 65 to 28, phenobarbital sodium 24 to 17, and diphenylhydantoin sodium variably. Duration of activity of these anticonvulsants was also increased. The following results will be discussed: 1- The difference in percentage protection at various dose levels of the anticonvulsants in the presence or absence of iproniazid. 2- The increased duration of anticonvulsant activity induced by iproniazid.

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Pharmacology

THE PHARMACOLOGY OF BETA-PHENYLISOPROPYLHYDRAZINE. Joseph P. Buckley, Angelo R. Furgiuele* and William J. Kinnard*. Department of Pharmacology, University of Pittsburgh School of Pharmacy, Pittsburgh, Pa.

The effects of beta-phenylisopropylhydrazine HCl (PIH) and iproniazid on spontaneous and forced motor activity were investigated in male, albino Swiss mice. The compounds were administered intraperitoneally in doses ranging from 2-75 mgm./kgm. of PIH and 5-480 mgm./kgm. of iproniazid. Low doses of PIH elicited a slight reduction in spontaneous activity, however doses greater than 10 mgm./kgm. produced stimulation, with a maximum increase in activity of almost 400% occurring with 20 mgm./kgm. Iproniazid produced a marked depression of spontaneous activity in doses over 10 mgm./kgm. Chronic administration of PIH to rats and dogs produced symptoms resembling ataxia and skeletal muscle relaxation. The effects of PIH on the flexor reflex of dogs was investigated to determine the possible mechanisms of this reaction. Ten mgm./kgm. was administered I.V. every 20 minutes. Facilitation of the reflex usually occurred with the first or second doses followed by a gradual depression and finally complete blockade between the 3rd and 12th dose. It appears that the hind limb weakness and ataxia was probably due to selective depression of spinal polysynaptic pathways. (Supported by USPHS Grant MY-3029, and Lakeside Laboratories Inc.)

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Pharmacology

A NEW NON-HYDRAZIDE MONOAMINE OXIDASE INHIBITOR (A19120) (N-METHYL-N-BENZYL-2-PROPYNYLAMINE HYDROCHLORIDE). J. D. Taylor, A. A. Wykes*, Y. C. Gladish*, W. B. Martin* and G. M. Everett. Abbott Labs., N. Chicago, Ill.

A19120 is one of a series of potent monoamine oxidase (MAO) inhibitors. In vitro it is 7 times more potent than iproniazid as measured by O_2 uptake of rat liver mitochondria with serotonin as substrate. O_2 uptake, NH_3 production and serotonin disappearance indicate a direct effect on the MAO. This compound exhibits properties of irreversible MAO inhibitors. A single oral dose of 200 mg/kg in mice inhibited MAO in liver and brain for at least 10 days. This correlated well with the increased irritability and activity produced by 200 mg/kg dl-DOPA given I.P. The oral LD_{50} of A19120 is 750 mg/kg. Doses of 100 mg/kg orally in the dog produce, after a delay of several hours, gradually increasing irritability, mydriasis and salivation lasting 24 hours or longer. Smaller daily doses given to rats over a period of 4 months have shown no adverse effects. These animals show a MAO inhibition of 90% and 80% in brain and liver, respectively.

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Pharmacology

ACTIVITY OF VARIOUS PHARMACOLOGICAL AGENTS AS IN VIVO INHIBITORS OF MONOAMINE OXIDASE. David H. Tedeschi, Ralph E. Tedeschi, and Edwin J. Fellows. Research and Development Division, Smith Kline and French Labs., Philadelphia, Pa.

Evidence was presented in a previous publication (J. Pharmacol. & Exper. Therap., 126: 223, 1959) that potentiation of the convulsant action of tryptamine serves as a useful measure of in vivo monoamine oxidase (MAO) inhibitory activity. The present report is concerned with the in vivo MAO inhibitory activity of a variety of pharmacological agents tested by this technique. All drugs were tested orally, unless otherwise specified, at their time of peak activity. Drugs effective as in vivo MAO inhibitors (arranged in descending order of potency) included harmine (s.c.), tranylcypromine (SKF trans 385), SKF cis 385, d-α-methylphenethylhydrazine, d,l-α-methylphenethylhydrazine, harmine (oral), d,l-N-acetylmethionylisopropylhydrazide (RO-4-1018), nialamide, phenelzine, iproniazid, and l-isonicotinyl-2-phenylisopropylhydrazine. Compounds which failed to effect significant in vivo MAO inhibition included diphenhydramine, tripelennamine, isoniazid, dihydroergotamine, mescaline, procaine, procaine amide, β-dimethylam.noethyldiphenylpropylacetate (SKF No. 525-A), and 2-amino-1-(3,4-methylenedioxyphenyl) propane hydrochloride (SKF No. 5-A).

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Pharmacology

EFFECTS OF NIALAMIDE ON THE ELECTROENCEPHALOGRAM. William H. Funderburk and Anna B. Drakontides*, Research Laboratories, Chas. Pfizer & Co., Inc., Groton, Conn.

Cats treated with nialamide in daily doses of 10 mg/kg subcutaneously over a two to five day period were studied. In unanesthetized cats, nialamide did not produce EEG arousal, but persistent behavioral arousal became apparent within 24 hours. During behavioral arousal sleep spindles in the electrocorticogram, although at times poorly formed, occurred more frequently than in untreated animals. In contrast, there was at the same time a marked reduction of sleep spindles in the medial thalamus. Spontaneous electrical activity in the mesencephalic reticular formation was slowed, but the threshold for producing EEG arousal was not altered. The usual fast activity in the hippocampus occurred in short bursts following nialamide. Thresholds for after-discharges in the hippocampus and motor cortex were unchanged. Hippocampal after-discharges were consistently reduced, while cortical after-discharges remained the same. The presence of sleep spindles during behavioral arousal may be the result of sustained wakefulness due to repeated doses of nialamide.

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Pharmacology

BIOCHEMICAL ASPECTS OF THE ACTION OF NIALAMIDE. K. F. Finger, (intr. by J. A. Schneider.) Research Laboratories, Chas. Pfizer & Co., Inc., Groton, Conn.

The inhibition of monoamine oxidase (MAO) by nialamide (N-isonicotinyl-N'-(β-N-benzylcarboxamido-ethyl)-hydrazide) has been studied in the cat, dog, rat, and rabbit. Nialamide has been found to be an inhibitor of MAO in the heart, kidney, and spinal cord as well as the brain and liver. The in vitro pI_{50} is 4.3. Levels of brain norepinephrine (NE) and serotonin (5HT) were elevated by nialamide in all species except the cat where it produced a five to seven fold elevation of 5HT but no significant change in NE. Nialamide inhibits the deamination of dopamine as well as 5HT, and the influence of nialamide on dopamine levels is being evaluated. The administration of reserpine to nialamide pretreated animals failed to reduce the levels of 5HT or NE in the brain or heart. Despite inhibition of kidney and heart MAO, kidney NE was not elevated and only the rat showed elevations in heart NE. In contrast to its effect on MAO, nialamide had little or no effect on brain dopa decarboxylase, transaminase or carbonic anhydrase systems. These data suggest an important role for MAO inhibition in nialamide's action, but this concept appears to be insufficient in explaining all of the observed pharmacological properties of this drug.

12 *Pharmacology*

ROUTE OF ADMINISTRATION OF THE HYDRAZINES AS A DETERMINANT IN THE SELECTIVE INHIBITION OF BRAIN AND LIVER MONOAMINE OXIDASE. A. Horita. Dept. of Pharmacology, University of Washington School of Medicine, Seattle.

Several of the monoamine oxidase (MAO) inhibitors which are currently employed as psychic stimulants have been studied for their abilities to selectively inhibit brain or liver MAO when administered by various routes. The drugs were administered to rats by the subcutaneous (S. Q.), intraperitoneal (I. P.) and oral routes. Certain of these agents, such as Marsilid and Niamid, showed no difference in organ selectivity regardless of the route of administration; greater liver MAO inhibition occurred consistently. Others, such as Catron and Nardil, exhibited different degrees of brain and liver inhibition when given by different routes. These drugs showed relatively selective inhibition of brain MAO when administered S. Q., but when given orally showed predominant liver MAO inhibition. This change in organ selectivity with oral administration of these latter drugs seem to be associated with their initial uptake by the liver as they pass through the hepatic portal circulation. (Supported by a grant (M-2435) from the U.S.P.H.S.).

13 *Pharmacology*

RESERPINE PTOSIS IS UNRELATED TO BRAIN CATECHOLAMINES LEVELS. Irmino Costa and Gordon R. Pscheidt*. Galesburg State Research Hospital, Galesburg, Illinois.

Brodie, et al. (J. Pharmacol., in press) found that sedation induced by tetrabenzine is related to decreases of brain serotonin but not to those of catecholamines. The experiments to be presented are in agreement with this report. In rats given 2 mg/kg, i.m. of tranylcypromine (SKF 385) 1 hour after reserpine (2.5 mg/kg, i.p.) the onset of ptosis occurs later than in animals given reserpine alone. The brain catecholamine content of rats given both drugs is unrelated to the degree of ptosis. On the contrary, a positive correlation exists between eye closure and levels of brain serotonin. The onset of ptosis is also retarded by 2 mg/kg, i.m. of amphetamine given 1 hour after reserpine. In normal rats SKF 385 raises brain serotonin levels whereas amphetamine does not.

14 *Pharmacology*

DELAYED AROUSAL OF RESERPINE-TREATED RABBITS BY MONOAMINE OXIDASE INHIBITOR. S. Spector*, R. Kuntzman*, C. Hirsch* and B.B. Brodie. Natl. Insts. of Health, Bethesda, Md.

Rabbits were pretreated with reserpine (5 mg/kg) and 3 hours later were given 10 mg/kg JB 807 (1-phenylisopropyl-2-isopropyl hydrazine) or 1 mg/kg MO 911 (N-benzyl-N-methyl propynylamine HCl) potent MAO inhibitors which unlike JB 516 (Catron) do not exert an amphetamine-like effect. No observable behavioral change occurred for ten hours. However by 20 hours the animals had almost completely recovered from the effects of reserpine. In contrast the reserpine-treated control animals were still highly sedated. The brain levels of norepinephrine in the animals given reserpine followed by a MAO inhibitor remained depleted (0.06 μ/gm) over the time course of the experiments. The brain serotonin levels rose from 0.14 to 0.31 μg/g and the brain dopamine level from 0.00 to 0.04 μg/g but these were no higher in 20 hours than in 2 hours after giving the MAO inhibitor.

15 *Pathology*

EFFECT OF AMINE OXIDASE INHIBITORS ON E. COLI ENDOTOXIN MORTALITY IN MICE. R. K. Ausman, R. B. Davis, W. R. Meeker and D. G. McQuarrie (intr. by F. Hoffbauer). Depts. of Surg. and Med., Univ. of Minn. Med. School

Gordon and Lipton demonstrated a protective effect of serotonin against endotoxin mortality in mice. Davis et al found a marked fall in serum serotonin levels after administration of lethal doses of E. Coli endotoxin to dogs. The present experiments were designed to assess the influence of amine oxidase inhibitors, which inhibit serotonin breakdown, on mortality. Two groups of A strain mice were used. One group received 10 mg/kg betaphenylisopropylhydrazine intraperitoneally and challenged 2 hrs. later with doses of E. Coli endotoxin from 2-40 mg/kg. The other group received 0.2 cc saline and was challenged at the same dose levels 2 hrs. later. The mortality at each dose level was tabulated at 12, 24, 36, 48, and 72 hrs. for both groups and statistically analyzed by the method of Litchfield and Wilcoxon. PIH was found to exert a protective effect against E. Coli endotoxin mortality from 1.07 to 6.8 times that of saline. Data comparing the relative potency of amine oxidase inhibitors will be presented. (Supported by grants from the Minn. Heart Assn. and U. S. P. H. S.)

182. NEUROPHARMACOLOGY–Sedative Agents

1 *Biochemistry*

THIORIDAZINE EXCRETION PATTERNS AFTER SINGLE AND MULTIPLE DOSES. Edward Geller and Samuel Eiduson (intr. by Max S. Dunn.) Vet. Adm. Center, Los Angeles and Med. Center, Univ. of Calif. Los Angeles.

A patient with an outdwelling catheter was administered a single dose of thioridazine (Mellaril) and the amount and rate of excretion of the phenothiazine-like metabolites were determined in the urine. Fast (tablet) and slow (space-tabs) acting forms of medication were compared. The concentration of excreted metabolites was also measured during continuous dose medication over a long period of time. Urine collections were made every two hours and the concentration of unconjugated phenothiazine was determined (Eiduson and Wallace, Vet. Adm. Trans. 2nd Res. Conf. Chemotherap. in Psychiat., 2, 85, 1958). With a single dose of the tablet form of drug, the peak of excretion was attained at about 20 hours after administration, while the peak following the space-tab appeared about 40 hours after administration. Within approximately one week, no drug could be detected in the urine (below 20 μg/10 ml urine). On continuous dose, the peak urinary excretion appears between 2 to 3 weeks; it then levels off. It was further observed that the phenothiazine metabolites are excreted in the urine in an oscillatory manner from one 2 hour period to another. The slow acting form of medication appears to be excreted in a manner similar to the usual tablet form.

2 *Pharmacology*

KINETICS OF PENTOBARBITAL PLASMA LEVELS IN THE DOG. R. G. Wiegand*, R. A. Krause*, K. Staniszewski* and J. D. Taylor. Dept. of Pharmacology, Abbott Laboratories, North Chicago, Ill.

The plasma level curve of pentobarbital in the dog can be interpreted in terms of three constants corresponding to apparent first order absorption, apparent first order disappearance from plasma and the apparent volume of distribution. Eight fasted dogs were given pentobarbital by oral solution, average dose 30.7 mg Na salt/kg (26.3-35.2), and blood samples drawn at 1/2, 1, 2, 3, 4, 6 and 8 hrs. A curve calculated according to the equation $c = a_o k_a (e^{-k_d t} - e^{-k_a t}) / V'_d (k_a - k_d)$ was fit to the data, where c = plasma conc. in mg pentobarbital Na/l, a_o = dose in mg/kg, V'_d = apparent volume of distribution in l/kg, and k_a and k_d are the apparent first order constants for absorption and disappearance from plasma in reciprocal hours. An estimate of the std. dev. of the values of k_a, k_d and V'_d which give the best fit of the curve to the average plasma levels was obtained by the least squares method applied to the average observed and calculated plasma levels. The values of the constants so determined were $k_a = 4.0 \pm 0.12$ hr^{-1}, $k_d = 0.08 \pm 0.0025$ hr^{-1} and $V'_d = 1.32 \pm 0.012$ l/kg. This data indicates that pentobarbital is absorbed and metabolized by first order kinetics, and that it is concentrated in tissues or spaces other than plasma, which is in agreement with tissue/plasma ratios measured directly.

3

Pharmacology

ANTICONVULSANT ACTIVITY OF A METABOLITE OF PHENOBARBITAL. Charles R. Craig*, Kaneo Hirano* and Frederick E. Shideman, Dept. of Pharmacology, Univ. of Wisconsin, Madison.

The lack of parallelism between hypnotic and anticonvulsant activities among the barbiturates and the insignificant hypnotic activity of 5-ethyl-5-(p-hydroxyphenyl) barbituric acid (p-OH phenobarbital) led us to examine this metabolite of phenobarbital for anticonvulsant activity. The ED_{50}s for maximal electroshock seizure (MES), minimal electroshock threshold (MET), hyponatremic electroshock threshold (HET), and metrazol seizure (Met) tests, toxic dose50 (TD_{50}) and LD_{50} (mg/kg, i.p.) were determined in the mouse for phenobarbital and its metabolite by standard methods (J. Pharm. Exp. Therap. 106: 319, 1952). PI (Protective index), TD_{50}/ED_{50}, is indicated by the figure in parentheses immediately below the ED_{50} value for that test.

	LD_{50}	TD_{50}	ED_{50} for MES	MET	HET	Met
phenobarbital	235	50	20	25	18	25
			(2.5)	(2.0)	(2.8)	(2.0)
p-OH phenobarbital	1920	1275	368	262	160	445
			(3.5)	(4.9)	(8.0)	(2.9)

Although less active than the parent compound the metabolite possessed significant anticonvulsant activity and its PI in each test was greater than that for phenobarbital.

4

Pharmacology

THE PHARMACOLOGY OF SOME PHENOSELENAZINES. Jerome N. Glassman, Albert J. Begany,* Marvey H. Bless,* George N. Hudyma* and Joseph Seifter. Wyeth Inst. Med. Res., Radnor, Pa.

It has been found that Se can be substituted in the phenothiazine nucleus to form compounds retaining many characteristics of promazine or chlorpromazine. The phenoselenazines resemble promazine and chlorpromazine in cardiovascular and adrenolytic action, antihistaminic, hypoglycemic, barbiturate potentiating potential and for protecting against epinephrine-induced pulmonary edema. The ataractic potency however is considerably reduced. On the other hand, oxidation of the phenothiazine nucleus to the sulfoxide or sulfone results in loss of ataractic and hyperglycemic potential; moreover, animals are not protected against epinephrine-induced pulmonary edema.

5

Pharmacology

THE INFLUENCE OF RESERPINE AND BULBOCAPNINE ON TONIC EXTENSOR SEIZURES IN MICE. Graham Chen and Barbara Bohner*. Res. Div., Parke, Davis and Co., Detroit, Mich.

Both reserpine and bulbocapnine produced facilitation of pentylenetetrazol-induced tonic extensor seizures in mice. The previous administration of iproniazid prevented the effect of the former but not that of the latter drug. Although bulbocapnine itself could not easily induce tonic-extensor seizures by intravenous infusion in mice, an extensor seizure response to the drug occurred under the influence of reserpine. However, intraperitoneal injection of a lethal dose of bulbocapnine would result in pentylenetetrazol type convulsions: viz. clonic seizures, followed by extension of limbs and death. Whereas reserpine could lower the extensor seizure threshold to electroshock, bulbocapnine like pentylenetetrazol was ineffective. Thus, bulbocapnine appears to have a similar action as pentylenetetrazol in the production of tonic extensor seizures. Reserpine, on the other hand, causes facilitation of tonic extensor seizures by a different mechanism; it is probably due to the depletion in the central nervous system of some biogenic amines which possess an anti-extensor seizure activity.

6

Pharmacology

THE EFFECT OF CERTAIN NEUROPHARMACOLOGIC AGENTS ON BRAIN ACETYLCHOLINE. Giancarlo Pepeu* and Nicholas J. Giarman, Yale University, Sch. of Med., New Haven, Conn.

A number of depressants and stimulants of the central nervous system (CNS) were studied for their influence on the level of total acetylcholine (ACh) in the whole brain of the rat. In general, it was substantiated that elevation of ACh accompanied CNS depression, while CNS excitation was associated with a decline in ACh. The following CNS depressants, in single doses, led to increases in ACh of from 17 to 90 percent, in this descending order of potency: METHYLPARAFYNOL ('Dormison'); HYDROXYDIONE SUCCINATE ('Viadril'); MORPHINE; PENTOBARBITAL; and RESERPINE. Chronic administration of reserpine (0.5 mg./kg., daily) led to an increased level of ACh on the second day, but to no change in subsequent days (up to 8), despite the progressive depression. When reserpine was administered to animals kept at 4° C., some failed to show the characteristic depression, and brain ACh in these animals was unaltered. The level of ACh was not modified, under these experimental conditions, by cortisone, LSD-25, or iproniazid ('Marsilid'). Studies to elucidate the mechanisms of these effects are being carried out on choline acetylase and cholinesterase activity, and on the intra-cellular distribution of ACh. (Supported by grant B-940 from National Institute of Neurological Diseases and Blindness).

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Pharmacology

DIFFERENTIAL EFFECTS OF PHENOBARBITAL, PENTOBARBITAL AND DIPHENYLHYDANTOIN ON MOTOR CORTICAL AND RETICULAR THRESHOLDS IN MONKEYS. Roy Aston* and Edward F. Domino, Dept. of Pharmacology, University of Michigan, Ann Arbor.

Phenobarbital has been suggested to be a useful anticonvulsant by virtue of a specific depression of the motor cortex (Keller and Fulton, 1931). To evaluate this hypothesis it was decided to study the effects of intravenous phenobarbital sodium (Phb), pentobarbital sodium (Peb) and diphenylhydantoin sodium (Dph) on the thresholds of electrical stimulation of the motor cortex (MC) and mesencephalic reticular formation (RF) of the monkey. Five animals with chronically-implanted electrodes were studied. Linear equations were established for the regression of voltage threshold on dose (mg/kg) in all cases except that of Dph on the RF, where no dose-response relationship was evident. Estimated slopes for MC were Phb 0.07, Peb 0.15 and Dph 0.04 and for RF were Phb 0.04 and Peb 0.17. Doses required to raise the control threshold for MC by 50% were Phb 19.9, Peb 10.8 and Dph 35.6 mg/kg. Equivalent threshold-elevating doses for RF were Phb 48.6 and Peb 12.4 mg/kg. The data indicate that Phb and Dph elevate the motor cortical threshold in doses that do not significantly depress the reticular formation. On the other hand, Peb elevates both thresholds at similar dose levels. (Supported by grant R-14-59C United Cerebral Palsy Research and Educational Foundation, Inc.)

8

Pharmacology

THE INHIBITION OF ALCOHOL DEHYDROGENASE BY CHLORPROMAZINE. Lip Boen Khouw*, T. N. Burbridge and A. Simon*. Depts. of Pharmacology and Psychiatry, Univ. of California. School of Medicine, San Francisco.

During a metabolic study of problem drinkers, it was observed that chlorpromazine increased the blood alcohol level (J. Appl. Physiol., In Press). To analyze the cause, a study was carried out in rabbits. It was found that the sole cause of the blood level elevation was an inhibition of the conversion of ethanol to acetaldehyde. In this study, horse liver alcohol dehydrogenase (ADH) was isolated by a slight modification of a previously described method (Bonnichsen and Brink, "Methods in Enzymology," Vol. I, Edited by Colowick and Kaplan). Measurement of activity was achieved by determining the reduction of DPN to DPNH spectrophotometrically. The Michaelis constant of the isolated ADH compared favorably with previously determined values. The addition of chlorpromazine strongly inhibits the enzyme at a concentration of 2.8×10^{-6} M (1 microgram/ml.). Both promethazine and promazine also inhibit at a similar concentration but the inhibition is not as great. (Supported in part by a grant from the Division of Alcoholic Rehabilitation, State of California, Department of Public Health).

183. NEUROPHYSIOLOGY

1 *Physiology*

EFFECTS OF PARTIAL DECENTRALIZATION ON THE NEUROMUSCULAR SYSTEM OF THE RAT. J.J. Seguin* (intr. by G.W. Stavraky) Dept. of Physiology, Faculty of Medicine, University of Western Ontario, London, Canada.

The excitability and contractility of the nerves and muscles were studied in decerebrated white rats 7 to 120 days after semisection of the spinal cord at T-2 level. Kymographic, EMG and nerve potential recordings of responses of the quadriceps, tibialis and gastrocnemius nerve-muscle preparations to electric stimulation of the severed nerve showed that the previously decentralized nerve-muscle preparation had a lower threshold than the control. This was found to be due in part to a lower threshold of the nerve itself and in part to an increased muscular excitability as determined in isolated preparations and in situ. Eserine (0.2-0.8 mg./kg. I.V.) and adrenaline (0.02-0.04 mg./kg. I.V.) increased the responses of the nerve-muscle preparation more on the semisected side, whereas mild asphyxia of the animal or of the muscle itself as well as ether anesthesia depressed responses more markedly on the side of the semisection. The results indicate that a partially decentralized neuromuscular system becomes more sensitive to excitatory and depressant influences than a fully innervated one. (Supported by the Muscular Dystrophy Association of Canada and the National Research Council of Canada).

2 *Physiology*

PROJECTIONS OF THE PREFRONTAL LOBE IN MONKEY. June L. DeVito and Orville A. Smith, Jr. (intr. by T. C. Ruch) Depts. of Anatomy and Physiology & Biophysics, Univ. of Washington School of Med., Seattle, Wash.

A portion of the prefrontal lobe anterior to a line joining the tips of the upper and lower limbs of the arcuate sulcus was removed bilaterally or unilaterally. The monkeys with bilateral lesions showed increased locomotor activity. The period to sacrifice varied from 7 days to 6 weeks. The tissues were prepared with the Nauta stain for degenerating axons. Neither the caudate nucleus nor the olfactory tract was involved in the lesions. The cingulum, the uncinate fasciculus and the subcallosal fasciculus showed heavy degeneration. Degenerating fibers of small diameter were present in the head of the caudate nucleus. The subcallosal gyrus and the septum pellucidum contained degenerated fibers. A well-defined band of degenerating fibers entered the pre-optic area. In the thalamus degeneration was present in the reticular, anterior ventral and dorsomedial nuclei. Degenerating fibers in the internal capsule passed into the medial portion of the peduncles and continued into the pons where terminal degeneration appeared throughout the medial pontine nuclei. No degeneration was apparent in the medullary pyramids. [Supported in part by a contract (Nonr 477(07)) with the Office of Naval Research.]

3 *Physiology*

FURTHER ANALYSIS OF PARAMEDIAN LOBULE RESPONSES TO CORTICAL AND BRAIN STEM STIMULATION. E. M. Housepian* and D. P. Purpura (intr. by P.F.A. Hoefer.) Dept. of Neurosurgery, College of Physicians and Surgeons, N. Y.

Extracerebellar origin of synaptic interactions conditioning 8-12 msec latency surface-positive components of paramedian lobule (PL) responses is indicated by changes in activity cycles produced by strychnine, nembutal and asphyxia in conjunction with variations in site of origin of cerebellopetal volleys. In decerebrate cats weak medial pontomesencephalic CS produce short latency (< 2msec) long-duration (40-60 msec) facilitation (> 300%) of bilaterally evoked PL responses. Stronger CS sufficient to evoke CR produce late developing inhibition of testing responses (TR). With weak CS, CR is markedly augmented by asphyxia and nembutal (5-15 mg/K) and undergoes cyclic changes after strychnine. Subsequent changes in TR are dependent on induced changes in CR. Facilitatory interactions may occur in contralateral PL and inhibitory in homotopic regions of ipisilateral PL. Such interactions, prominent in PL responses to pericruciate cortex stimulation are rarely observed following stimulation of brachium pontis or caudal pontomedullary reticular regions.

4 *Physiology*

PROGRESSIVE INCREASE OF ELECTROSHOCK CONVULSIVE THRESHOLD IN CATS. Carl F. Essig and Harold G. Flanary.* NIMH Addiction Research Center, USPHS Hospital, Lexington, Kentucky.

The current necessary to reproduce human electroconvulsions gradually increases (Kalinowsky, L., Res. Publ. Ass. Nerv. Ment. Dis., 26: 179, 1947). Several aspects of this phenomenon in cats are reported herein. An insulated platinum electrode was fixed in the skull over each hemisphere. A Grass S-4 stimulator was used to deliver current in 3-volt increments every 5 minutes until a convulsion occurred at threshold. Two msec biphasic pulses at 200/sec were delivered for 5 seconds. Current was determined by using the IR drop across a 100-ohm resistor. Three cats were convulsed 95 to 163 times during 48 to 155 days. Initial thresholds varied from 5.0 to 8.0 and eventually increased to levels between 9.0 and 15.0 milliamperes representing elevations of 44 to 46%. Thresholds fell to original levels within one to five weeks of discontinuing daily stimulation. Spontaneous convulsions developed in one cat after having 142 electrical seizures; a phenomenon which disappeared when stimulation was stopped. Histological studies indicate the same minor alterations in cerebral cortex at the electrode sites in both a stimulated and a non-stimulated cat. It is postulated that the elevation of threshold (tolerance ?) depends on a reversible biochemical adaptation rather than anatomical alterations. The spontaneous seizures may prove less dependent on threshold elevation than total number of induced convulsions.

5 *Physiology*

PERFUSION OF CEREBRAL VENTRICULAR SYSTEM IN UNANESTHETIZED GOATS. J. R. Pappenheimer, J. deC. Downer and J. N. Nicholl, Jr.* Harvard Med. School, Boston, Mass.

Large syringe needles are placed through skull so that tips lie 1-2 mm. above a lateral ventricle and the cisterna magna and hubs protrude 2 cm. above skin. The needles are fixed permanently to skull. After recovery from operation the ventricle and cistern may be punctured at will with #17 needles inserted 3-4 mm. beyond tips of the fixed, implanted tubing. Perfusion from ventricle to cistern with sterile, synthetic CSF can thus be carried out in unanesthetized animal. Resistance to flow is less than 1 cm. H_2O/ml/min. Rates up to 5 ml/min are tolerated without discomfort for many hours; perfusions may be repeated in same animal over period of months. Intracranial distribution volume of various tracers is about 10 ml. but volume varies with pressure. Outflow usually exceeds inflow in proportion to net formation of CSF. Two types of problems are studied 1) Exchanges between CSF perfusate and blood 2) Functional effects of changes in composition of perfusion fluid. Anatomical and temperamental characteristics make goats specially suited for this type of study. (Supported by a grant from A.H.A.)

6 *Physiology*

ACTION OF ADRENALIN AND ESERINE ON ADULT MAMMALIAN BRAIN CULTURES. (MOTION PICTURE) R.S. Geiger, C. Adachi, and M. Stewart (intr. by P. Bailey.) Research Laboratories, Dept. of Psychiatry, Univ. of Illinois, Chicago.

Using time lapse microphotography--on the addition of adrenalin (0.001-0.005 µgr/ml) to cultures of brain cortex formation of neurofibrils can be observed. These fibrils appear first at the nucleoli and then spread into the cytoplasm. Effects on nuclear cytoplasmic interaction, Nissl substance and synaptic areas are demonstrated. Oligodendroglia show increased rate of pulsation and secretion; astrocytes increased twitching. Effects on cultures of subcortical white matter are also shown. Eserine sulfate (0.00001-0.0001 µgr/ml) markedly effects the cell surface and synaptic areas. A visible movement of mitochondria down the axon occurs. Evidence of increased oxidative metabolism in synaptic areas is presented. (Supported by Mental Health Fund, State of Illinois Dept. of Public Welfare.)

7 *Physiology*

ELECTRICAL CONNECTIONS BETWEEN SUPRAMEDULLARY NEURONS. M.V.L. Bennett (intr. by R.M. Taylor). College of Physicians & Surgeons, Columbia U., New York.

Polarization of one supramedullary neuron produced in nearby neurons slower and smaller polarizations that were apparently the result of electrotonic spread. Spread occurred in either direction between two cells and was not an electrical artifact, since it was observed with differential recording and disappeared when the polarizing microelectrode was removed to an extracellular position. Maximum spread from soma to soma was about 1/6th. The electrical connections probably mediate synchronization of responses to synaptic stimulation and spread of excitation on stimulation of a single cell (J. Gen. Physiol., 43: 159, 1959). The previous data indicate that both synchronizing connections and synapses of afferent fibers occur along the axons. In confirmation of the axonal site, synaptically evoked responses recorded in efferent axons were only partially blocked and desynchronized by complete removal of the cell bodies. Furthermore, electrotonus between two cells was not prevented by destruction of an intervening band of cell bodies.

8 *Biochemistry*

Effect of O-methylation and Other Substitutions of Indoles on Lightening of Frog Melanocytes. James D. Case and M. Ruth Wright (intr. by George R. Cowgill.) Yale Univ. School of Med., New Haven, Conn.

Recent efforts in this laboratory have led to isolation of melatonin, an agent from beef pineal glands which causes melanin granule aggregation in frog melanocytes. Its structure is 5-methoxy-N-acetyltryptamine. To study structural requirements for lightening activity about forty synthetic indoles were obtained with various substitutions of the indole nucleus, particularly at positions 3 and 5. These compounds were tested by reflection photometry for ability to lighten the previously darkened surviving skin of Rana pipiens. Nearly all compounds lightened at some concentration. However, a 3-aminoethyl side chain and oxygen at positions 5 were required for high activity. Melatonin was unique, needing 10^{-12} to 10^{-13} grams/ml. for measurable lightening. Potencies compared with melatonin included: 5-ethoxy N-acetyltryptamine 10^{-1}, 5 methoxy N-formyltryptamine 10^{-3}, 5 hydroxy N-acetyltryptamine 10^{-4}, 5 methoxytryptamine 10^{-2}, serotonin 10^{-7}, N-acetyltryptamine 10^{-5}, tryptamine 10^{-6}, norepinephrine 10^{-6}, acetylcholine 10^{-6}. We conclude that a wide variety of indoles lighten frog skin, but O-methylation and N-acetylation of serotonin are necessary for maximum effect.

9 *Pharmacology*

FACTORS AFFECTING CILIARY ACTIVITY ON THE GILL OF THE MUSSEL. Edward Aiello (intr. by D. Lehr.) Dept. of Physiol. and Pharmacol., New York Medical College, Flower and Fifth Avenue Hospitals, New York City.

The known stimulatory effect of veratrine on ciliary activity was studied in greater detail. In the presence of veratrine, rate of beating and velocity of the metachronal wave increased; average wave length remained constant. Oxygen uptake increased. Oxygen was found to be required for prolonged or vigorous beating and in its absence veratrine had only a transient effect. Ciliary activity was inhibited by cutting the branchial nerve. Activity could be restored by adding veratrine. Serotonin and a hot water extract of gill tissue gave all the effects of veratrine. The active agent in the extract has not yet been identified but it has been found to have a serotonin-like stimulating effect on the clam heart and the rat stomach. It is tentatively suggested that this agent may play a role in maintaining ciliary activity in situ. (Aided by grant No. B-2497 (A) from the Natl. Inst. Health, PHS.)

10 *Pharmacology*

IMPAIRMENT OF CATECHOLAMINE BINDING SITES IN BRAIN BY RESERPINE. R. Kuntzman and S. Spector (intr. by B.B. Brodie). Natl. Insts. of Health, Bethesda, Md.

It has been shown that reserpine impairs the ability of brain tissue to bind serotonin, so that the stored amine is released and metabolized. Since brain catecholamines are also released, we investigated whether reserpine also impairs the ability of brain tissue to bind catecholamines. Dopa, 10 mg/kg, was given to normal and reserpinized rabbits. In both normal and reserpinized animals brain dopamine levels increased rapidly and reached a maximum in about 5 min. In the normal animals the level decreased slowly whereas, in reserpinized animals, the level decreased rapidly. For example, 30 minutes after dopa injection there was an increase of .32 γ/gm dopamine in normal rabbit brain compared to only .14 γ/gm in reserpinized rabbit brain. When a monoamine oxidase inhibitor was given before administration of dopa, the dopamine increase in the brain of normal and reserpinized rabbits was the same. This indicates that reserpine does not inhibit the formation of dopamine in vivo, but impairs the ability of the brain to store this catecholamine.

11 *Pharmacology*

NOREPINEPHRINE AND SEROTONIN BRAIN LEVELS AT VARIOUS STAGES OF ONTOGENETIC DEVELOPMENT. N.T. Kärki*, R. Kuntzman* and B.B. Brodie. Natl. Insts. of Health, Bethesda, Md.

Newborn rats, which have poorly developed patterns of behavior, have low levels of brain norepinephrine (NE) and serotonin (5HT). Three days before birth, NE is about 15%, 5HT about 25% of normal (10 week old rats). At birth, NE is 20% and 5HT 50% of normal. Two weeks later NE and 5HT levels were 40% and 70% respectively of normal; in seven weeks they approached the values of the mature animal. Newborn guinea pigs, which in contrast to newborn rats display well developed patterns of behavior, have brain levels of both NE and 5HT almost as high as those of mature guinea pigs (12 weeks old). Activities of dopa and 5HTP decarboxylase and of monoamine oxidase have also been determined at various stages of development.

184. NEUROPHYSIOLOGY–Acetylcholine

1 *Physiology*

THE BRAIN ACETYLCHOLINE-ACETYLCHOLINESTERASE-CHOLINE ACETYLASE SYSTEM IN RESPIRATORY CONTROL. Bernard Metz. Medical College of South Carolina, Charleston, S.C.

The significance of neurohumoral factors (e.g. acetylcholine) in respiratory control remains uncertain. Experiments indicate a significant correlation between respiratory function (as measured by the reflex response to electrical stimulation of Hering's Nerve) and the acetylcholinesterase (AChE) activity and acetylcholine (ACh) concentrations in representative brain areas, after injection of the anticholinesterase, tetraethyl pyrophosphate (TEPP), via cisternal puncture. With a decreasing respiratory reflex there is a gradual decrease in the AChE activities in the brain areas, accompanied by a corresponding progressive increase in the ACh concentrations in these areas. No correlation was evident, however, between the choline acetylase activities and the respiratory reflex responses. Subsequent experiments utilized the oximes, pyridine-2-aldoxime methiodide (PAM) and 2-hydroxyiminomethyl-N-methylpyridinium methanesulphonate (P2S), to reactivate the AChE activity after it had been inhibited by TEPP, and analyses of the ACh-AChE-Choline Acetylase system additionally support the possibility of a cholinergic factor in central respiratory control. (Supported by a grant from the Natl. Insts. of Health, B-954).

2 *Physiology*

ROLE OF THE ACETYLCHOLINE SYSTEM IN CONDUCTION. W.D. Dettbarn (intr. by D. Nachmansohn.) Dept. of Neurol. College of P. & S., Columbia University, New York.

Ranvier nodes of single frog sciatic nerve fibers have been exposed to several compounds known to react with the acetylcholine system. The effects on electrical activity have been studied. Eserine, a strong inhibitor of acetylcholinesterase, reversibly blocks conduction in concentrations of 10^{-4} M (about 30-40 μg/ml). In smaller concentrations it increases the spike amplitude and prolongs its descending phase. Prostigmine, an equally strong inhibitor of acetylcholinesterase, blocks conduction in a tenfold higher concentration. d-Tubocurarine which blocks synaptic transmission also rapidly blocks axonal conduction when used in concentrations between 10^{-3} M and 5×10^{-3} M, it also increases the spike duration. The effects of these three compounds are readily reversible. Paraoxon which combines irreversibly with acetylcholinesterase blocks conduction in 10^{-3} M. This effect is irreversible.

3 *Biochemistry*

BIOELECTRIC ACTIVITY RESTORED BY CHOLINESTERASE REACTIVATORS. L.P. Hinterbuchner (intr. by D. Shemin) Dept. of Biochem., Coll. of P. & S., Columbia U., N.Y.

When Cholinesterase (Ch.E.) falls below 20% of the initial value, conduction in excitable tissues is blocked. Electrical activity (E.A.) once irreversibly blocked by alkylphosphates cannot be restored by washing. Restoration has been attempted with specific Ch.E. reactivators. Two strips of muscle from frog sartorius were dissected, one for control. Denervated muscles were also used in order to enhance permeability to reactivators. Muscles were exposed to paraoxon, one strip washed with Ringer, the other with reactivator. 2-PAM proved ineffective. However, the more lipid soluble benzoyl derivatives of PAM were more successful. In 5 of 11 exps. there was excellent restoration of E.A., in 4 there was moderate improvement. Restoration of E.A. by specific Ch. E. reactivators emphasizes the essentiality of this enzyme for conduction. The action potential frequently increased in amplitude both during the exposure to paraoxon, before inhibition, and in the recovery phase of reversible inhibition, supporting the postulated association of acetylcholine with conductance changes.

4 *Pharmacology*

STEREOSPECIFICITY OF SPECIFIC CHOLINESTERASES. B.V. Rama Sastry and J.V. Auditore (intr. by M.T. Bush.) Depts. of Pharmacology and Medicine, Vanderbilt Univ. School of Med., Nashville, Tenn.

The reaction kinetics of the hydrolysis of L(+)-, D(-)- and DL-lactoylcholines (LCh), and of acetylcholine (ACh) by specific (human RBC) cholinesterase have been studied and activity-pS curves have been compared. The activity-pS curve for DL-LCh is bell-shaped and resembles that of ACh. The activity-pS curve of DL-LCh for the enzymic hydrolysis showed a higher value at pS optimum (1.9) than that of ACh (1.3). The ratio between the reaction velocities for ACh and DL-LCh was 0.15 at pS 1.3 and 2.0 at pS 1.9. At pS 1.3, L(+)-LCh was hydrolyzed at a rate five times faster than that of D(-)-LCh. This indicates that specific cholinesterase distinguishes between these enantiomorphic pairs of choline esters containing an asymmetric carbon in the alpha position to the carbonyl group. The relative molar activities of lactoylcholines for muscarinic and nicotinic effects correlate with the rates at which the isomers are hydrolyzed, in vitro, by human specific cholinesterase. The D(-)-isomer which is more active in muscarinic and nicotinic effects is hydrolyzed at a slower rate than the L(+)-isomer which is less active in muscarinic and nicotinic effects.

185. NEUROPHYSIOLOGY–Amine Oxidases

1 *Pharmacology*

EFFECT OF MONOAMINE OXIDASE INHIBITORS ON DIAMINE OXIDASE (HISTAMINASE). V.H. Cohn, Jr.* and P.A. Shore. Natl. Insts. of Health, Bethesda, Md.

Iproniazid has been reported to inhibit the action of diamine oxidase (DAO) as well as monoamine oxidase (MAO) in vitro. To examine the effect of iproniazid in vivo, we administered iproniazid, 100 to 200 mg/kg (as base) intraperitoneally to rats and measured intestinal DAO activity (using histamine as substrate) of rats killed at various times. DAO activity was markedly inhibited within one hour and was still inhibited 24 and 48 hours later although iproniazid has been shown to have disappeared from tissues after 24 hours. Thus the action of iproniazid on DAO as well as MAO appears to be "irreversible". Testing the ability of various compounds to inhibit guinea pig liver MAO and DAO activity in vitro showed that at 10^{-4} M, MAO inhibitors containing a hydrazine moiety -- iproniazid, JB 516 (phenylisopropylhydrazine), Ro 4-1634 (1-benzyl-2-trimethylacetylhydrazine), Ro 4-1038 (1-seryl-2-isopropylhydrazine) -- inhibited DAO, while MAO inhibitors not possessing the hydrazine moiety -- SKF 385 (phenylcyclopropylamine) and SKF 556 (N,N-diethyl-2-phenylcyclopropylamine) -- did not inhibit DAO. Other hydrazine containing compounds at 10^{-4} M (hydralazine, aminoguanidine, isoniazid) inhibited DAO but not MAO. A corresponding pattern of inhibition was obtained in vivo.

186. NEUROPHYSIOLOGY–Autonomic N S

1 *Physiology*

SUPRASPINAL POSTTETANIC FACILITATION OF GALVANIC SKIN REFLEX IN ANESTHETIZED CATS. G. H. Wang. Dept. Physiology, Univ. Wisconsin, Madison, Wis.

In cats under chloralose (50 mg/kg) and urethane (1 g/kg), 5 sec stimulation of the anterior hypothalamus first suppresses the galvanic skin reflex (evoked by stimulation of the central end of a divided cutaneous nerve) during the first minute after cessation of stimulation and then facilitates the reflex to a great extent. The facilitation lasts for 20 – 40 minutes. Stimulation of the midline area of the dorsal surface of the dorsal thalamus for the same duration has similar effects on the galvanic skin reflex. In cats under the same anesthesia but with the cerebral peduncles isolated after precollicular decerebration, 5 sec stimulation of one peduncle also has similar effects on the galvanic skin reflex, but the duration of facilitation is shorter than that of stimulation of either the hypothalamus or the dorsal thalamus. Stimulation of the sigmoid gyrus or the infralimbic area of the cerebral cortex has no posttetanic facilitatory effect on the reflex. The practical use and the theoretical significance of the observations will be discussed. (Supported by grant B-1460, NINDB.)

2 *Physiology*

BIOASSAY OF 5-HYDROXYTRYPTAMINE ON HEART OF VENUS MERCENARIA. A.R.McIntyre, David E. Williams* and F.L.Humoller* Univ. of Nebraska College of Medicine Omaha Nebraska.

Threshold sensitivity of the Venus heart to concentrations of 5-HT somewhat below 10^{-10} are well established. With concentrations from 10^{-8} to 10^{-9} the heart amplitude varies linearly with log concentration. With dilutions greater than 10^{-9} responses as indicated by amplitude changes become difficult to duplicate. With greater concentrations than 10^{-7} successive doses evoke smaller responses which tend to decrease progressively or vanish; resembling tachphylaxis. When solutions are adjusted, after preliminary trial, to concentrations of 10^{-8} to 10^{-9} assays on unknowns with an error of ± 10% are obtained. For best results the heart should be suspended so that the contiguous segment of gut lies transversely and not parallel with the contracting axis of the heart. Standard solutions must be applied immediately preceding or immediately following the solutions undergoing test and should be reasonably close to the anticipated concentrations undergoing assay. It should be noted that the simultaneous presence of ACh and 5-HT in biological fluids may invalidate the assay of either substance. However ACh can be readily removed by hydrolysis and 5-HT is relatively stable.

XUM

3

Pharmacology

MYDRIATIC HALF-LIFE OF A NEW ANTICHOLINERGIC AS AFFECTED BY DOSE, ROUTE, AND QUATERNIZATION. F.M. Sturtevant. Smith Kline & French Labs., Philadelphia 1, Pa.

If the degree of a pharmacologic response is determined by the number of receptors occupied, then the rate of disappearance of response should be equal to drug concentration at the target times some constant. The reciprocal of this constant is the metabolic half-life, which is calculated from a straight line plot of log pharmacologic response versus time. The apparent half-life was determined for mydriasis in mice produced by the new tertiary ammonium anticholinergic SKF-5515, 9-methyl-3-oxo-9-azabicyclo-(3,3,1)-nonan-7-yl benzilate maleate (Wilfon & Macko, Fed. Proc. 18: 458, 1959) and the methobromide quaternary derivative SKF-5516, following i.v., s.c., and p.o. administration. Maximal response appeared to depend upon the number of absorptive barriers traversed by each drug; SKF-5516 was inconsistently absorbed orally. The apparent half-life of SKF-5515 was independent of dose and route within the limits of experimental error ($T\frac{1}{2}$ = 2 hrs.). However, the apparent half-life of SKF-5516 was dependent upon log dose and route, possibly as a result of decreased facility of passage across membrane barriers. These results may be illustrated in terms of the kinetics of the metabolism of drugs.

4

Pharmacology

THE BRADYCARDIA OF CHRONIC CHOLINERGIC BLOCKADE, David Jerram* and R. P. Ahlquist, Dept. Pharmacology, Med. Coll. of Ga., Augusta, Ga.

We have reported that chronic cholinergic blockade (CCB) produced by daily administration of adequate doses of atropine and related compounds reduces the tachycardia evoked by acute administration of the same substances; heart rates were 11 to 29% lower in 12 of 13 dogs. To study this effect, the blood pressure and heart rate (HR) responses to 90° tilt, hexamethonium (C6), epinephrine, isoproterenol and ethylnorepinephrine were determined before and after CCB by atropine in a series of dogs. Acute atropine tachycardia was reduced in each case. The blood pressure changes during and after tilt were not altered by CCB. The HR continued to show a decrease during the rise in pressure at the end of the tilt period, but the anticipated HR increase during tilt did not occur. The ganglion blocking action of small doses of C6 was not potentiated by CCB. Blood pressure and HR changes produced by epinephrine, isoproterenol and ethylnorepinephrine were not consistent, but in some animals the HR increase was greatly reduced. Thus the reduction in acute atropine tachycardia is due to a veratramine- or dichloroisoproterenol-like blockade. The HR levels during CCB support the latter interpretation. (Supported by grant H-3514, NHI, USPHS.)

5

Pharmacology

SUPERSENSITIVITY TO NOREPINEPHRINE AFTER PRETREATMENT WITH RESERPINE. W. W. Fleming* and U. Trendelenburg, Dept. of Pharmacology, Harvard Med. School, Boston, Mass.

The relation between the sensitivity of the nictitating membrane (n.m.) to norepinephrine (NE) and the depletion of NE stores has been studied in reserpine-pretreated spinal cats. Depletion was estimated by comparing the response of the n.m. to continuous nerve stimulation with its response to continuous infusions of graded doses of NE. The sensitivity of the n.m. was determined by dose response curves from intravenous injections of NE. First series: A single intraperitoneal injection of reserpine (0.1, 0.3, 1 and 3 mg/kg) 24 hr prior to the experiment caused increasing depletion but no supersensitivity. Second series: A daily dose of 0.1 mg/kg reserpine was given for 1, 3, 7, and 14 days. Depletion increased with time and total dose and reached about the same level as in the first series. Some supersensitivity was apparent after 7 and 14 days, but it was much less pronounced than that observed after denervation or cocaine. The results indicate that there is no direct relationship between depletion and supersensitivity. Although reserpine causes severe depletion within a day, time is essential for the development of supersensitivity to NE. (Supported by U. S. Public Health Service Grants B-1713 and 2G-95.)

6

Pharmacology

SUBSENSITIVITY TO CERTAIN SYMPATHOMIMETICS AFTER PRETREATMENT WITH RESERPINE. U. Trendelenburg and W. W. Fleming*, Dept. of Pharmacology, Harvard Med. School, Boston, Mass.

The relation between the sensitivity of the nictitating membrane (n.m.) to tyramine (T) and to ephedrine (E) and the depletion of the norepinephrine (NE) stores has been studied in spinal cats. The sensitivity of the n.m. was determined by dose response curves from intravenous injections of T or E. Reserpine pretreatment caused pronounced depletion and usually no supersensitivity to NE (see Fleming and Trendelenburg, this meeting); it abolished the response of the n.m. to T; the response to E was reduced, but by increasing the dose, nearly maximal responses were still obtained. The observations agree with the view that T may normally act by releasing NE from the stores (and its action is thus abolished by reserpine-pretreatment), whereas E may have a mixed action: its direct effect on the n.m. is reserpine-resistant, whereas its indirect effect is abolished by reserpine. The results suggest that the phenomenon of subsensitivity is directly related to the depletion of the NE stores and can be separated from the phenomenon of supersensitivity to NE. (Supported by U.S. Public Health Service Grants B-1713 and 2G-95.)

7

Pharmacology

GANGLIONIC "BLOCKADE" BY NICOTINE. E. W. Pelikan and George C. Garcia.* Dept. of Pharmacology, Boston Univ., Boston, Mass.

Data were obtained consistent with the hypothesis that nicotine (N) may prevent ganglionic transmission by impairing acetylcholine release (Fed. Proc. 18:432, 1959). A modified Kibjakov preparation of the superior cervical ganglion was studied in 22 barbitalized cats. Venous effluent was collected from the posterior transverse facial vein; in only 1 cat could the internal jugular vein be cannulated. Acetylcholine was assayed on the rat ileum. In 3 out of 3 experiments, intra-arterial injection of N (100 mcg) was followed by decreased acetylcholine output in venous effluent and abolition of nictitating membrane response to preganglionic stimulation. There was little relation of acetylcholine output to preganglionic stimulation, but perfusion of the dead ganglion yielded no acetylcholine. In none of the cats could blood-free effluent be recovered while the cat lived; loss of perfusate into the cat could not be prevented. Injection of N increased resistance to perfusion, decreased the flow of effluent, but increased the fraction of infused fluid recovered as effluent. With the Kibjakov preparation, effects of drugs on the vessels perfused make studies of acetylcholine output from the preparation difficult to interpret.

8

Pharmacology

EFFECT OF TM-10, COCAINE AND RESERPINE ON ISOPROTERENOL VASOMOTOR REVERSAL. D. T. Walz*† and G. D. Maengwyn-Davies. Dept. of Pharmacology, Georgetown Univ. Schools of Medicine and Dentistry, Washington , D. C.

l- and dl-Arterenol were found to inhibit isoproterenol reversal by phenylephrine. This was substantiated in chronically reserpinized dogs. Since TM-10 and cocaine have been suggested to act as agents blocking the release of norepinephrine from its depots, the effect of these drugs on isoproterenol reversal by phenylephrine was further studied. It was found that the phenylephrine reversing dose could be substantially reduced both after TM-10 as well as after cocaine. After TM-10, cocaine no longer potentiated the isoproterenol pressor responses, which are potentiated by cocaine in controls. Ephedrine in the reserpinized dog reversed isoproterenol at much lower doses than in the normal animal. Since ephedrine has been shown to act by norepinephrine release, this further substantiates the inhibitory action of norepinephrine on this phenomenon. (Supported by Grant B-1448, National Institute of Neurological Diseases and Blindness, U.S.P.H.S.) († Postdoctoral Research Fellow, BF-9183, NINDB)

9 *Pharmacology*

GANGLIONIC STIMULATING EFFECTS OF BUTYRYL-BETA-METHYLCHOLINE. Frederick F. Cowan[1] (intr. by T. Koppanyi). Dept. of Pharmacology, Georgetown University Medical and Dental Schools, Washington, D. C.

The effects of butyryl-beta-methylcholine, recently synthesized by M. Wurzel, have been compared with those of other choline esters on the blood pressure of anesthetized dogs and on the nictitating membrane contractions of anesthetized cats. If an appropriate time lapse is allowed between doses in stropinized cats, using a modified Acheson technique, butyryl-beta-methylcholine causes contractions of the nictitating membrane by stimulation of the superior cervical ganglion. This compound markedly potentiates itself. Potentiation by physostigmine, therefore, is difficult to demonstrate after control runs. The responses are inhibited by hexamethonium. After initial stimulation of the superior cervical ganglion, butyryl-beta-methylcholine induces a transient blockade of transmission to intermittent stimulation of the preganglionic fibers. In the non-atropinized animal, butyryl-beta-methylcholine has a potent peripheral action. Benzoyl-beta-methylcholine, another new compound tested, in contrast, shows far less ganglionic stimulating properties. ([1] Postdoctoral Fellow, HF-9232, National Heart Institute, U.S.P.H.S.)

10 *Pharmacology*

THE EFFECTS OF AMOBARBITAL SODIUM AND MEPROBAMATE ON AUTONOMIC REACTIVITY AS MEASURED BY CARDIOVASCULAR RESPONSES TO THE COLD PRESSOR STRESS. K. Rickels, J.H Ewing and F. Fochler (intr. by C.J. Lambertsen). University of Pennsylvania School of Medicine, Phila.

An inverse relationship between syst. bloodpressure(BP) rize under coldpressor(CP) stress and syst. BP drop after methacholine inj. has been demonstrated recently. The syst. BP response in both tests was felt to depend on central autonomic reactivity. To study the effects of 2 centrally acting, anxiety-reducing agents, 15 subjects were tested under standard CP stress, 2 hrs after randomized oral doses of placebo, amobarbital Na (200mg/60kg), and meprobamate (400 mg). BP and heartrate were recorded. In contrast to placebo, syst. BP baseline was lowered by both drugs while diastolic baseline was slightly raised with meprobamate. CP stress produced significantly less rize of syst. and diast. BP under meprobamate than under placebo or amobarbital Na. No corralation existed between BP baselevels and BP reaction to the CP stress. Under both drugs,(but more under amobarbital Na.), baselevel heartrate(h.r.) was lowered and CP stress produced a larger increase of h.r. than under placebo.

11 *Pharmacology*

BLOCKADE OF ADRENERGIC INHIBITORY RESPONSES OF THE ISOLATED RABBIT INTESTINE. Bert K. B. Lum and Perry L. Rashleigh (intr. by Lawrence Peters), Kansas University Medical School, Kansas City, Kansas.

The purpose of these experiments was to study the effect of several adrenergic blocking agents on the responses of the rabbit intestine to inhibitory agents. Epinephrine, phenylephrine, norepinephrine, isoproterenol and nitroglycerin were administered in approximately equi-effective concentrations to the isolated rabbit jejunum preparation. Dihydroergotamine (DHE) and yohimbine in low concentrations effectively antagonized the responses to epinephrine and phenylephrine. The responses of the other agents were not modified or required higher concentrations of DHE and yohimbine for blockade. The dichloro analogue of isoproterenol (Lilly 20522) inhibited the response to isoproterenol but not to those of the other agents. It is postulated that these results reflect variations in drug affinity for multiple adrenergic receptor sites.

12 *Pharmacology*

SENSITIZATION OF THE NICTITATING MEMBRANE TO SYMPATHOMIMETIC AMINES BY RESERPINE. Ian R. Innes (intr. by M. Nickerson). Dept. of Pharmacology & Therapeutics, U. Manitoba, Winnipeg.

The hypothesis of Burn & Rand (J. Physiol., 147:135, 1959) that supersensitivity of denervated smooth muscle is due to loss of normal noradrenaline stores is based on evidence that supersensitivity accompanies noradrenaline depletion due to reserpine or postganglionic denervation. The hypothesis has been tested by use of the established difference in the patterns of sensitization following preganglionic and postganglionic denervation. After preganglionic denervation adrenaline remains several times more potent than noradrenaline, whereas after postganglionic denervation these amines are equipotent. Reserpine, within 5 min. of i.v. injection in cats, before depletion of noradrenaline stores could occur, enhanced the responses to both amines. Continued recording for 16 hr. showed gradually increasing sensitization. Cats injected with reserpine for 2-10 days prior to study showed marked supersensitivity. In all experiments adrenaline remained considerably more potent than noradrenaline, resembling preganglionic rather than postganglionic denervation. To conform to the hypothesis of Burn and Rand the pattern of sensitization should resemble that of postganglionic denervation, since the evidence is against the loss of noradrenaline content after preganglionic denervation.

13 *Pharmacology*

RESERPINE INDUCED ALTERATIONS IN THE REACTIVITY OF AN ADRENERGIC EFFECTOR SYSTEM. B.L. Mirkin and P. Cervoni (intr. by R.F. Furchgott) State Univ. of N.Y., Brooklyn, N.Y.

Investigations have been initiated to determine whether the responsiveness of a typical adrenergic neuro-effector synapse to autonomic blocking agents may be altered by prior treatment with reserpine. The response of the cat's nictitating membrane was recorded isometrically. Electrical stimulation was applied to the post-ganglionic cervical sympathetic nerve fibers. Reserpine (5 mg/kg) was administered i.p. at least 24 hours prior to each experiment. Contractile responses induced by nerve stimulation in the reserpinized animals appeared to be refractory to blockade by phentolamine(P). The mean depression was 18% of control levels, whereas blockade in non-reserpinized animals ranged from 62-82%. In contrast, atropine(A) was markedly effective in the reserpinized animal (61% decrease), as compared to the non-reserpinized animal (18% decrease). The response to exogenous catecholamines and cholinergic agents was blocked by (P) and (A) respectively. The effectiveness of (P) and (A) in blocking the responses of the nictitating membrane to nerve stimulation in normal and reserpinized animals appears to be reversed. These data suggest that an adrenergically-dominated system is altered to one in which cholinergic mediation assumes the predominant role. (Supported by Grant #H-3054, Heart Inst.,NIH; Life Insur.Med.Res.Fund.)

187. NEUROPHYSIOLOGY–Brain

1 *Physiology*

THE EFFECT OF CEREBRAL EDEMA ON THE THIOCYANATE SPACE OF RAT BRAIN. Eugene Streicher and Gilbert D. Press (intr. by James E. Birren). Section on Aging, National Institute of Mental Health, Bethesda, Maryland.

It has generally been assumed that in the brain as in other tissues edema results in an enlargement of the extracellular compartment. The basis of this assumption lies in the fact that water, sodium and chloride analysis of human autopsy material as well as edematous nervous tissue of experimental animals indicates that the "excess fluid" corresponds fairly closely with an ultrafiltrate of plasma. However, the bulk of brain sodium and chloride may actually be localized intracellularly, since estimations of the size of the extracellular space by means of electron microscopy or the distribution of thiocyanate yield values of less than 10%. The thiocyanate space of edematous rat brain was measured to ascertain whether exogenous NaCl from the plasma enlarged the space or was apparently distributed in the same manner as the endogenous ions. Since no increase in the volume of distribution of thiocyanate was observed in experimental animals, these studies suggest that edema of the brain may be primarily an intracellular process.

2 *Physiology*

MITOCHONDRIAL CHANGES IN DEVELOPING RAT BRAIN. Frederick E. Samson, Jr.,, William M. Balfour* and Roberta J. Jacobs. Dept. of Physiol., Univ. of Kansas, Lawrence.

The cerebral energy requirement increases during neonatal maturation with a corresponding increase in mitochondrial protein. The question arises: Do cerebral mitochondria (M) change in number or in size during this period? M prepared by differential centrifugation from rats of known ages were counted with phase contrast microscopy, also the dry weight and protein determined. The number of M per gram (g) of brain is $(12)(10^{10})$ in 1-day rats, $(30)(10^{10})$ in 21-day rats, $(33)(10^{10})$ in 50-day rats. The average dry weight in $\mu\mu$g of a M is 0.387 in 1-day; 0.309 in 21-d; 0.288 in 50-d. The protein is 0.154 in 1-d; 0.114 in 21-d; and 0.104 in 50-d. Thus it appears there is a 3-fold increase in the number from 1 to 21 days but the mass per M decreases. There is little change in number from 21 to 50-d and the mass per M continues to decrease. Calculation from the known total dry weight and protein increase per gram of brain shows that 75% of dry weight and 61% of the new protein synthesized is M. (Aided by Nat'l Insts. of Health, Grant B-1151 and Post-doctoral Fellowship)

3 *Physiology*

DIFFERENTIAL LOCALIZATION OF TASTE AND TONGUE TACTILE AFFERENTS IN THE RAT THALAMUS. Raimond Emmers, Robert M. Benjamin and Murray F. Ables. (Intr. by W.I. Welker) Dept. Physiology, Med. School, Univ. of Wisconsin, Madison.

The thalamus was mapped electrophysiologically for potentials evoked by two types of stimuli: 1) electrical stimulation of the taste nerves (chorda tympani and IXth) and 2) tactile stimulation of the tongue. Responses were confined to a morphologically distinct subnucleus in the most ventral and posterior part of the n. ventralis. The taste nerves projected to the medial half of this subnucleus: the chorda tympani, anteriorly; the IXth posteriorly. Tactile responses were confined to the lateral half of the subnucleus with only slight overlap into the taste nerve area. It was concluded that the medial part of the subnucleus was devoted exclusively to taste. The taste function of this medial part was confirmed by other experiments which demonstrated that destruction of this medial area alone produces severe and permanent impairment of taste discrimination. (Supported by grants from NIMDB, B-1932 and B- 732).

4 *Physiology*

BRAIN AND SOCIAL BEHAVIOR IN THE MONKEY (16 mm. color moving picture with sound) José M. R. Delgado
Yale University School of Medicine, New Haven, Connecticut

The purpose of this film is to demonstrate techniques to quantify social behavior of monkeys and to study modifications induced by drugs and by brain stimulation. The first part explains the methods used: (i) to record social behavior by time-lapse photography, (ii) to analyze and quantify the records by means of a time-study projector, electric typewriter, and electronic computer, and (iii) to program stimulation of the animals with tiny transistor-stimulators carried on a collar and connected to the brain through subcutaneous leads. The second part of the film shows quantification of 24 categories of daily behavior. Administration of chlorpromazine, reserpine, iproniazid, pentobarbital and estradial modify the quality and quantity of behavioral categories. Stimulation of central gray increases the aggressiveness of the stimulated monkey and produces social spread of attacking behavior. Stimulation of motor areas does not disturb the animals. Stimulation of the nucleus anterior ventralis of the thalamus evokes running away and hiding. Histological study shows that prolonged stimulation of the brain does not alter the neurons. (Supported by grants from the Office of Naval Research and U. S. Public Health Service).

5 *Physiology*

WIRELESS REMOTE-CONTROL MINIATURE MICRODRIVE FOR SINGLE NEURON RECORDING IN THE BRAIN. Felix Strumwasser and James J. Gilliam, (intr. by Choh-Luh Li), N.I.H., Bethesda, Maryland

The valuable technique of a remote-control hydraulic microdrive system, for recording single neuron activity in the brain of unrestrained, unanesthetized cats, has been recently described by Hubel (J.P. 147: 226, 1959). We will describe a miniature electromechanical microdrive specifically developed for such recordings in smaller animals. The microdrive weighs 7 grams, holds either glass or metal microelectrodes, has a range of fine drive of 7 mm., but permits unlimited total depth of penetration and resolves to approximately 0.2μ in reproducible steps. The microdrive system consists of a small magnet mounted on a worm, which, through a miniature 1250:1 reduction gear arrangement, moves a 1/4 mm. lead screw carrying the microelectrode. The magnet is controlled remotely by a pulsed or rotating magnetic field applied to coils positioned outside the animal's cage. Rather simple shielding techniques permit recording while the drive is in motion. The electromechanical microdrive system has certain advantages over the hydraulic system: smaller size and weight, greater resolution and reproducibility, unlimited depth of penetration, wireless control and ability to hold glass micropipettes. We wish to thank Messrs. Bak and Ziminsky for design of the two-phase power oscillator controlling the rotating magnetic field.

6 *Physiology*

ALTERNATION OF THE PRIMARY CORTICAL RESPONSE EVOKED BY PERIPHERAL NERVE STIMULATION. James Frost*, Peter Kellaway, and Meyer Proler*, Baylor Univ. Coll. of Medicine, Houston, Texas

Forbes, et al, 1949, showed that 9/second sciatic stimulation produced "alternation" of the evoked cortical response so that every second response was absent or reduced. This effect has been re-investigated to localize its origin and determine the mechanism. After a period of alternation the even numbered responses progressively increase in voltage while the odd responses decrease until at a critical point each stimulus is effective and elicits an equal response. During this equilibration the odd responses have a longer rise time than do the even, the latency remaining constant. At threshold nerve stimulation the evoked response alternates for a longer period and shows a longer latency than with maximal stimuli. Alternation can occur at different rates in two or more adjacent regions of the sensory cortex independently. The effect is confined to each individual projection and does not involve the sensory area as a whole. Stimulation of the medial lemniscus produces alternation at the cortex and internal capsule but not in the PVL nucleus or medial lemniscus. Stimulation of the PVL nucleus or internal capsule, however, produces non-alternating responses. Alternation occurs in the capsule after removal of the cortex. Thus, the mechanism of alternation is localized at a thalamic level.

7 *Pathology*

THE PATTERN OF PROTEIN TURNOVER IN THE MOUSE BRAIN AT REST AND AFTER STIMULATION, STUDIED WITH C^{14} LABELED SUBSTANCES AND RADIOAUTOGRAPHY IN SERIAL SECTIONS. **J. Beuttas, M. Balaban, and A. Geiger (intr. by Orville T. Bailey.) Research Laboratories, Dept. of Psychiatry, Univ. of Illinois, Chicago.**

The brains of mice killed 40 to 80 minutes after the injection of tracer substances were fixed, sectioned and coated with photosensitive nuclear emulsions. Various tracer substances and coating methods were tried and their relative merits are discussed. Uniformly C^{14} labeled leucine was found most suitable. Between injection and killing, some animals were resting; others were convulsed or stimulated via sensory nerves. The incorporation of C^{14} into the proteins of various parts of the brain is evaluated semi-quantitatively by counting of silver grains. Grain-density is evaluated in relation to cell-density, Nissl substance and physiological activity of the respective areas. Changes in C^{14} incorporation caused by electro-shock convulsions and by stimulation of selected nervous pathways are described.

188. NEUROPHYSIOLOGY–Brain–Brain Stem

Physiology

PYRAMIDAL CORTICIFUGAL DISCHARGES ELICITED BY IPSILATERAL CUTANEOUS STIMULATION. Harry D. Patton. Dept. of Physiology, University of Washington School of Medicine, Seattle, Washington.

In chloralose-decamethonium anesthetized cats single Betz cells (identified by antidromic pyramidal stimulation) were usually (69 of 85) driven by cutaneous shock to either contralateral (CFP) or ipsilateral (IFP) forepaw. IFP response latencies (mean 34.0 msec.) were always longer than those to CFP (mean 20.0 msec.). Multiunit responses to both CFP and IFP were also recorded by wire electrodes in bulbar pyramid. CFP responses often displayed two components, early (10-12 msec.) and late (25-35 msec.). IFP responses also showed early (15-20 msec) and late (25-35 msec.) components. Latency of early IFP component exceeds that of early CFP component by callosal conduction time (Area I to contralateral pyramid=4-5 msec.). Early IFP component is differentially abolished by callosal section. Data suggest two paths for IFP responses: a). early component relayed from contralateral to ipsilateral cortex via corpus callosum b). late component mediated by slowly conducting ascending path projecting to both cortices. (Aided by grant B-395, USPHS.).

2

Physiology

The Effect of an Organic Buffer (THAM) on Respiration, Arterial Blood pH and Respiratory Gases in Decerebrate, Pontile and Medullary Cats. R. L. Katz*, S. H. Ngai, G. G. Nahas and S. C. Wang. Depts. of Anesth. and Pharmacol., Coll. of Physicians and Surgeons, Columbia Univ., New York City.

In decerebrate, pontile and medullary cats THAM (trishydroxy methylaminomethane) was infused intravenously to study its effects on respiratory movements, arterial pH, P_{CO_2} and O_2 saturation. In the decerebrate preparation with eupnea 0.8 molar THAM infused at a rate of approximately 0.3 mM/kg/min to a total dose of 5 mM/kg produced hypopnea or apnea. The arterial blood pH rose. The P_{CO_2} first decreased and then rose with time. In the pontile vegotomized animal with apneustic breathing the cycling decelerated with marked prolongation of the inspiratory phase, pH rose and P_{CO_2} decreased. In the medullary cats with periodic breathing, the rate decreased while pH rose and P_{CO_2} decreased. These results agree with the previous hypothesis that the rate of cycling of apneustic breathing depends upon P_{CO_2} and/or pH. These results further suggest that the medullary preparation is capable of responding to changes in pH and P_{CO_2} in terms of rate and depth of respiretion.

3

Physiology

INFLUENCES FROM THE CAROTID SINUS UPON CORTICAL EVOKED POTENTIALS. W. P. Koella, C. K. Levy*, J. R. Smythies* and J. S. Czicman*. Worcester Found. for Exper. Biol., Shrewsbury, Mass.

In a previous study we had shown by "physiological fractionation", that serotonin injected into the common carotid artery, exerts its effect upon the cerebral sensory response through three receptor fields: the carotid sinus (factor x), the brain stem (y) and the specific sensory synapses (z). To demonstrate factor x directly, dial-anesthetized cats were prepared for recording of the cortical visual response. The normally innervated carotid sinus area was isolated from the circulation and canulated for perfusion with Ringer solution. Depending upon the dose, serotonin added to the perfusion fluid induced enhancement or reduction of the amplitude of the evoked response. With doses ranging from 10×2^{-9} to 10×20 µgm. a dose-response curve similar to the one calculated in the previous series was found. Furthermore, the time course of the changes in potential amplitude was similar to the one in the previous series.

4

Physiology

EFFECT OF LESIONS IN MEDULLA ON OCULOMOTOR RESPONSES TO TECTAL AND CORTICAL STIMULATION. Jane E. Hyde (intr. by D.B. Lindsley.) Dept. Physiol., UCLA Med. Ctr., Los Angeles.

Conjugate horizontal oculomotor responses to stimulation of superior colliculus or occipital cortex can be depressed or abolished by chronic or acute lesions in the medulla at and posterior to the level of the 6th nucleus (minimum size of lesions 10 mm^3). Of 5 cats lesioned 3 or more weeks prior to stimulation in the encéphale isolé preparation, eye movements from left occ. cortex required high voltage (i.e., were depressed) compared with those from right cortex in cats #2 and 5. Responses from left sup. coll. were similarly depressed in cats #2 and 3. Differentiation between cortically and tectally evoked eye movements was also seen in 5 acute experiments with 2 to 4 hindbrain lesions per cat. 3 of the 16 lesions in right medulla depressed cortically evoked responses; 3 lesions blocked responses to stimulation of left sup. coll. without altering cortical movements. Thus while oculomotor impulses from both the occ. cortex and the sup. coll. must reach the level of the contralateral medulla before activating the 3rd and 6th nuclei, extensive lesions at this level can selectively depress one type of induced eye movement while leaving the other unaltered. (Supported by grant B-1000 from the Public Health Service.)

5

Physiology

CHANGES IN ACTIVITIES OF RESPIRATORY NEURONS PRODUCED BY STIMULATION OF MUSCLE AFFERENTS. **K. Koizumi, J. Ushiyama* and C. McC. Brooks. Department of Physiology, State University of New York, Downstate Medical Center, Brooklyn, N. Y.**

Repetitive stimulation of muscle afferents produced an increase in ventilatory volume of anesthetized cats. This effect was observed also when ventral roots were severed and the stimulus was just strong enough to evoke a monosynaptic reflex. Activities of units within the medullary center were recorded by KCl-filled or indium-filled glass capillary microelectrodes. Inspiratory neurons generally showed a greatly augmented discharge frequency on stimulation of gastrocnemius afferents; they fired earlier when the expiratory phase of the cycle was shortened and even began to discharge during expiration as a result of this stimulation. Activities of other cells normally discharging in association with expiration were either augmented or depressed by stimulation of muscle afferents. Some of the expiratory and inspiratory units found to be affected by peripheral nerve stimulation ceased to discharge when respiration was suddenly stopped but others continued their rhythmic firing. (Supported by a grant from the National Institute of Neurological Disease and Blindness PHS-B-847 (C4).

6

Physiology

ELECTROCORTICOGRAMS OF CATS WITH CHRONIC LESIONS OF THE ROSTRAL MESENCEPHALIC TEGMENTUM. David H. Hubel and W.J.H. Nauta.* Walter Reed Army Institute of Research, Washington, D.C. In 7 cats electrolytic lesions were made at the mesodiencephalic boundary, extending forward to include part of the thalamus. In the first postoperative week cats were catatonic but apparently awake, yet showed continuous high voltage slow waves. Painful stimuli evoked low voltage fast activity. At times the cats adopted a sleeping posture and showed sporadic muscle twitching: slow waves were then replaced by low voltage rapid activity. These results support findings in normal cats suggesting that sleep in which low voltage rapid waves and twitching occurs is deeper than the high voltage slow state. After one week behavior became more normal, and cortical rhythms were mainly of the low voltage rapid type. Paroxysmal bursts of bilaterally synchronous 3-5/sec wave and spikes were now seen every few minutes, each burst lasting 2-10 seconds. Sometimes bilateral twitching of face muscles accompanied the wave-and-spike complexes. Attacks could be triggered by intermittent photic stimulation and abolished by natural arousal stimuli; spontaneous movements were absent during the attacks. By 2-3 weeks the attacks had ceased. It is concluded that a temporary condition in some ways resembling human petit mal epilepsy can be produced in the cat by a destructive brain stem lesion.

7 *Physiology*

MODIFICATION OF PERIPHERALLY EVOKED CUNEATE RESPONSES BY CEREBELLAR STIMULATION. A. M. Stoiber and C. D. Barnes. (intr. by H. N. Harkins.) Dept. of Physiology & Biophysics, Univ. of Washington School of Med., Seattle, Washington.

Cuneate neurons were isolated by the usual extracellular methods in cats anesthetized with Nembutal and paralyzed with Syncurine. The conditioning volley was either a single pulse or a 312/sec. train of pulses delivered via bipolar electrodes to the ipsilateral hemisphere or the anterior lobe of the cerebellum. The testing volley was a single 0.1 msec. shock to the ipsilateral forepaw; the intensity of this stimulus was varied from threshold to 100 times threshold. Cerebellar hemisphere shocks inhibited activity in all units isolated, and anterior lobe shocks inhibited activity in about half of them. The amount of interaction varied with the strengths of the conditioning and testing shocks, and the effects were best seen with weak testing and strong conditioning stimuli. The signs of interaction were a reduction in response probability, a reduction in the mean number of spikes per discharge, a significant increase in initial spike latency, and a slight increase in interspike interval. The interaction was fully developed in 20 msec. and disappeared in 150-200 msec. (Supported by grants 2-B5082 and B396 from the National Institute of Neurological Diseases and Blindness, USPHS.)

8 *Physiology*

HABITUATION TO DIRECT STIMULATION OF THE RETICULAR FORMATION. Stephen E. Glickman and Samuel M. Feldman. (intr. by A. A. Ward, Jr.) Dept. of Physiology & Biophysics, Univ. of Washington School of Med., Seattle, Washington.

Sharpless and Jasper (Brain 79:655, 1956) reported that the cortical EEG arousal produced by auditory stimulation habituates to iterative stimulation. In the present study, a 3-sec. train of square pulses (0.2 msec. duration, 0.5-10 V. amplitude, 250/sec.) was delivered to the nuclei of the upper brain-stem reticular formation in cats. The resultant arousal in chloralose-anesthetized animals had a brief latency (less than 0.2 to 1.5 sec.) and rarely outlasted the stimulus by more than 10 sec. Repetition of the stimulus train at intervals between 2 and 10 min. revealed a habituation which began after the first few trials and ultimately developed to such a degree that no noticeable arousal appeared. Habituation of the arousal response was also observed in unanesthetized preparations with chronically implanted electrodes. The duration of the unhabituated EEG response to direct stimulation of the reticular formation was greater in these animals than in anesthetized animals. The specificity of the habituation to the frequency and intensity of the arousing stimulus was studied in both acute and chronic preparations, as were the temporal characteristics of the habituation. (Supported by grant 2-B5082 from the National Institute of Neurological Diseases and Blindness.)

9 *Physiology*

MODIFICATION OF ACOUSTICALLY EVOKED CEREBELLAR RESPONSES BY SUBCORTICAL STRUCTURES. Charles K. Levy* and Paul Chervin* (Intro. by Fred Elmadjian). Worcester Found. for Exptl. Biol., Shrewsbury, Mass.

The effects of electrical stimulation of the reticular formation (RF) on the acoustically evoked cerebellar response were studied in chloralose anesthetized cats. Stimulating electrodes were placed in or near the diencephalic and mesencephalic reticular core by the Hess technique. The stimulating loci were localized by histological examination. The potentials evoked by loudspeaker clicks were recorded from the surface of the exposed vermal cortex in the conventional manner. Single shock stimuli to the R.F. evoked little or no response in the cerebellar acoustic projection area, but when these electrical stimuli were synchronously applied with acoustic stimuli the amplitude of both the positive and negative components of the evoked response was markedly enhanced. In addition to this interaction phenomenon prolonged changes (augmentation or inhibition) could be produced by repetitive electrical stimulation of the R.F. The effects of various stimulus parameters were studied (re. pulse duration, pulse frequency and pulse voltage). High frequency stimulation usually caused inhibition of the evoked response while low frequency stimulation of the same locus caused augmentation of the response.

10 *Physiology*

EFFECTS OF BRAINSTEM LESIONS ON VERTICAL GAZE IN MONKEYS. M.B.Bender and S.Shanzer,*Dept.of Neurol.Mt.Sinai Hosp.,N.Y.C.

Effects of small electrolytic lesions in monkey brainstem were evaluated in relation to direction of oculomotor defects produced. Such defects were found with lesions placed near the midline in the tegmentum of the brainstem. Unilateral lesions caused deficits in the horizontal plane. Manifest defects in the vertical plane were found only in animals with bilateral lesions. Bilateral lesions in the pretectum or upper midbrain caused palsies of vertical gaze only. Paralyses of upward and downward gaze or of isolated upward gaze were seen. Other effects included nystagmus in vertical plane, eyelid retraction and pupillary abnormalities. Small symmetrical lesions in the paramedian zone of pontine tegmentum, ventral to the MLF, resulted in paralysis of gaze in all directions, both vertical and horizontal. Isolated paralysis of downward gaze was thus far not found in this series. Vestibular stimulations normally inducing vertical nystagmus (rotation with the head in lateral position or simultaneous caloric stimulation of both ears) were performed in all animals. Appropriate nystagmus up or down could not be elicited in these animals with paralysis of vertical gaze. Monkeys with unilateral brainstem lesions never showed paralysis of vertical gaze but had defective responses in vertical plane upon bilateral vestibular stimulations.(Aided, in part, by USPHS Grant B-294.)

11 *Physiology*

CEREBRAL LOCI INVOLVED IN PENILE ERECTION. Paul D. MacLean and Detlev Ploog (intr. by R.B. Livingston) Natl. Insts. of Health, Bethesda, Maryland.

Squirrel monkeys are being used for stereotaxic exploration of brain to identify structures involved in penile erection. The subject sits peaceably in a restraining chair. Exploring electrodes are fixed at positive points for chronic study and determination of frequency-amperage-duration curves. Erections occurring optimally with stimuli at 30 per second have been obtained at sites histologically located in the septum, medial preoptic area, paraventricular nuclei, anterior thalamic group and mammillo-thalamic tract. Latencies vary from 3-7 seconds. Stimulations are frequently accompanied by potential changes in hippocampus and followed by hippocampal afterdischarges. Throbbing erections may wax and wane for periods up to 5 minutes following stimulation. Repeated stimulations seem to induce an enduring state of quietude that contrasts with agitation resembling fear or anger elicited from nearby structures. At points close to positive loci, urination or cardiac extrasystoles and/or cardiac slowing are commonly elicited. Erections occurring optimally with stimuli at 60-100 per second have been obtained from points along course of medial forebrain bundle and in region of subthalamic nucleus. Here the latencies are 3 seconds or less, and erections do not appreciably outlast the stimulation.

12 *Physiology*

INFLUENCE OF PERIODIC LUNG INFLATION ON RESPIRATORY PERIODICITY. Morton I. Cohen (intr. by W. W. Douglas). Albert Einstein College of Medicine, New York, N. Y.

In paralyzed cats with pneumothorax and intact vagi, the activity of the respiratory centers becomes synchronized with the periodic lung inflations produced by a positive-pressure respirator. Simultaneous recordings of intratracheal pressure and efferent phrenic nerve discharge show that the ratios of phrenic period and pump period are integers (2:1, 1:2, 1:3, 1:4). If the pump frequency is near the natural frequency of respiration, there is 1:1 correspondence; if the pump frequency is high, each phrenic discharge is synchronized with every second, third, or fourth inflation. Two principal modes of synchronization occur: (a) Each phrenic discharge starts before a lung inflation, and ceases towards the end of the inflation. (b) Each phrenic discharge starts immediately after the end of an inflation. Over a long series of cycles, the mode of synchronization may shift spontaneously between (a) and (b). These effects are dependent on vagal afferents, since after bilateral vagotomy the phase relation between phrenic cycle and pump cycle is random. The results can be interpreted in terms of the Hering-Breuer inflation reflex and of periodic activity within the respiratory centers.

13 *Physiology*

CLICK RESPONSES IN MESENCEPHALIC RETICULAR FORMATION DURING NATURAL SLEEP. Peter R. Huttenlocher (intr. by S. S. Kety.) Natl. Insts. of Health, Bethesda, Md.

Click responses in the mesencephalic reticular formation, the cochlear nucleus, and the primary auditory cortex were studied during sleep and wakefulness in cats with implanted electrodes. During the quiet waking state, click responses in the reticular formation consisted of slow waves of up to 150 μV amplitude which persisted undiminished after more than one thousand consecutive clicks. During sleep with EEG slow waves, habituation of responses in the reticular formation occurred after only 5-10 clicks. Responses became variable and mean amplitude decreased to about 50 per cent of response size in waking animals. During sleep with low voltage fast EEG activity there was almost complete suppression of brain stem responses to repeated clicks. Large responses returned immediately upon arousal of the animal. Click responses in the cochlear nucleus did not vary with changes in state of arousal. Responses on primary auditory cortex persisted during sleep except for a decrease in the late slow wave components. The results suggest that during sleep there is depression of evoked activity in the nonspecific afferent system, while activity in primary afferent pathways remains unchanged.

14 *Pharmacology*

THE EFFECT OF MORPHINE SULFATE ON THE CENTRAL RESPIRATORY MECHANISMS IN THE CAT. S. H. Ngai. Depts. of Anesth. and Pharmacol., Coll. of Physicians and Surgeons, Columbia Univ., N.Y.C.

In vagotomized midcollicular decerebrate cats the effect of morphine on the respiratory pattern and on the responses to electrical stimulation of various components of the respiratory center complex was studied. Morphine in repeated doses of 1-2 mg/kg given intravenously slowed the respiratory rate and caused either no change or an increase in tidal volume. Stimulus threshold of the medullary inspiratory center was elevated but the magnitude of the maximal response was not significantly altered. Stimulation of the pontile pneumotaxic center elicited inspiratory standstill instead of the usual acceleration and inspiratory shift. The acceleratory response upon low frequency (10-25 cps) stimulation of the central vagus was abolished. However, the expiratory standstill upon high frequency (100-200 cps) central vagal stimulation remained unchanged. In pontile decerebrate animals vagal sectioning after administration of morphine was followed by either slow apneustic breathing with expiratory phase predominating or slow deep periodic breathing. Levallorphan in dose of 0.5-1.0 mg/kg restored the usual apneustic pattern. These results indicate that morphine depressed the facilitatory function of the pneumotaxic center and vagal afferents. Although the inspiratory center is rendered less responsive the recruiting mechanism is apparently not impaired.

189. NEUROPHYSIOLOGY–Brain–Cerebral Cortex

1 *Physiology*

STUDIES ON THE SUPRACALLOSAL, MESIAL CORTEX OF CONSCIOUS, UNANESTHETIZED MONKEYS. J. R. Hughes, J. A. Mazurowski*, and J. E. Hauser*. Buffalo Med. School, Buffalo, N. Y.

Arrays of 50 electrodes, used for either stimulation or recording, have been implanted onto the supracallosal, mesial cortex of monkeys. Stimulation of all electrodes throughout the entire mesial surface in the conscious, unanesthetized animal elicits a specific movement. Representation of all body parts is found with head and facial areas at the poles and leg area in the middle. Yawning and piloerection have been elicited from the anterior, inferior mesial surface. Recording from within the olfactory bulb shows bursts of 35-40/sec. waves, seen only in the alert state and associated with respiratory inspiration. These fast waves are diminished in amplitude with most olfactory stimuli, except cigarette smoke which increases their amplitude. K-complexes are seen in the drowsy monkey and are elicited with sudden sensory stimulation, especially auditory. These K-complexes are seen prominently on three different regions, the anterior, mid and posterior cingulate areas. Evoked responses from photic stimulation have been recorded by the cathode-ray oscilloscope and the EEG machine. Responses are maximal in amplitude near the calcarine fissure and suddenly diminish in amplitude anterior to the region midway between the parieto-occipital fissure and the mesial extension of the central fissure. (Supported by a grant from USPHS-B1564)

2 *Physiology*

PERIPHERAL SENSORY FIELDS OF PYRAMIDAL TRACT (P.T.) CELLS. Vernon B. Brooks, Rockefeller Institute, New York, N.Y.

Spatial and modal origins of sensory input to P.T. cells are unknown. Experiments were made with 9 cats under local anesthesia and immobilized with flaxedil. 39 stable P.T. cells were identified in ant. and post. sigmoid gyri. All, except 5, could be driven either by hair bending and/or touch, or alternatively by pressure and/or joint movement. Excitatory levels increased with successive tests. At low levels, 30 cells had sensory fields only contralaterally, 5 ipsilaterally, and 4 on both sides. 'Unilateral' P.T. cells had slow 'spontaneous' discharge; and the sizes of their excitatory fields as well as the types of evoked discharge resembled those of 'sensory' cortical cells. Surround- and cross-modality-inhibition of P.T. cells were present. Repeated tests enlarged peripheral fields, activated previously inert modalities, and increased 'spontaneous' discharge. At the end of tests low excitability returned in secs. or mins. Thus activity of P.T. cells induced by peripheral physiological stimuli closely resembles that of sensory cells, except P.T. cells appear to be subject to summation from wider fields and more diverse modalities. Patterns of discharge, facilitation, and inhibition will be discussed.

3 *Physiology*

NICOTINE AND GABA ON CORTICAL TRANSMISSION. S. Ochs and K. Hunt*. Dept. Physiol. Ind. Univ. Med. School. Indianapolis, Ind

The possibility that nicotine and Gamma aminobutyric acid (Gaba) act as synaptic blocking agents in the cortex was indicated by their reversible block of propagation of spreading depression (SD). This was shown in the molecular layer preparation where SD transmission continues though all cortical layers below the first are cut through. Nicotine was placed either on the recording point while direct cortical responses (DCR's) were elicited or into a well-type of recording electrode while recording DCR's. It either had no effect on the DCR or augmented DCR's almost 2 fold. An increase in the DCR after nicotine application could come about by a selective block of inhibitory synapses acting on apical dendrites. Gaba either eliminated the DCR or reversed it and this was related to direct application or use of the well electrode. Gaba has been suggested as a specific blocking agent for excitatory synapses. SD block would then come about by block of inhibitory synapses (nicotine) or block of excitatory synapses (Gaba). Alternatively, nicotine may have an action on the release or reaction to transmitter substance postulated in the 'contiguity'theory of SD transmission. Gaba could also have this effect or more probably it acts in a generalized fashion on apical dendrites to interfere with SD and DCR transmission. (Supported by grants from PHI and NSF.)

4 *Physiology*

FAILURE OF INTERMANUAL TRANSFER OF TRAINING IN CORPUS CALLOSUM-SECTIONED CHIMPANZEES (16 mm color-sound movie). Ronald E. Myers (intr. by David McK. Rioch.) Walter Reed Army Institute of Research, Washington, D.C.

The corpus callosum transmits information between the hemispheres regarding the content of impinging visual stimuli so that visual learning achieved through one hemisphere is immediately available through the other. Does the great cortical commissure function similarly in relation to the content of somatosensory experience? This question was answered by training three normal and two corpus callosum and anterior commissure-sectioned chimpanzees to solve a latchbox problem with one hand and without visual aids. After they achieved rapid and stable solution with the one hand, they were required to solve the problem using the opposite, untrained hand. The normal animals promptly succeeded while the commissurotomized animals regularly failed and had to learn to solve the problem anew using the second hand. The same result obtained whichever hand received the initial training. The forebrain commissures are thus required for the exchange of information between the two hemispheres regarding somatosensory as well as visual learning experiences.

5 *Physiology*

A STUDY ON THE CAUSE OF BRAIN WAVES. Frederic H. Johnson (intr. by E.L. McCandless.) Chronic Disease Research Institute University of Buffalo, Buffalo, N.Y.

The electroencephalogram is said to be caused either by inherent rhythmic discharge of cortical neurons or by circuits between the cortex and subcortical structures. Electrolytic or mechanical lesions were made in sixteen cats with aid of stereotaxic instrument in subcortical structures on one side of the cat's brain with the unoperated side acting as control. Lesions were made from posterior approach in order that there would be no injury to the cortex. Areas removed were dorsal thalamus, basal ganglia, peduncular nuclei, and in case of cerebellar EEG the roof nuclei, and brachium. EEG records were read by Dr. F.A. Gibbs' laboratory (U.of Ill.). In all cases there is an absence or a very near absence (very low voltage) of brain waves on the operated side but electroencephalogram is present on the unoperated side. The situation is similar in cats which have recovered from ether narcosis. A few micro-volts of EEG are caused by the corpus callosum. The experiment confirms the theory of Papez that brain waves are caused by circuits between the cortex and subcortical structures. The theory that the electroencephalogram is caused by inherent rhythmic discharge of neurons is not supported by Burns' work (J. Physiol., 1950, 111: 50) because isolated cortex is rhythmically electrically inactive.

6 *Physiology*

CEREBELLAR INFLUENCES ON STEADY POTENTIALS AND SEIZURE ACTIVITY IN THE CEREBRUM. **T. Ozaki* and R. S. Snider. Northwestern Univ., School of Med., Chicago, Ill.**

Slow potential shifts were recorded by means of DC amplifiers from cats under ether and Flaxedil anesthesia from the pial surface of the cerebral hemisphere following induction of seizure activity (1 sec. to 2 min. duration) in the cerebrum by electrical stimulation. The duration of the seizure activity in addition to the duration and amplitude of the DC change could be influenced by simultaneous stimulation of the cerebellum or nucleus ventralis lateralis thalami. In case of the cerebellum, there were diminished DC changes and seizure activity while in the case of VL there seemed to be prolonged activity. In cats, following chronic removal of one cerebral hemisphere and recording the above mentioned activities from the other hemisphere there was a reversal of the above-described effects. For example, combined cerebrum and cerebellar stimulation prolonged the DC and the seizure activity, while combined cerebellar and ventralis lateralis stimulation shortened the effects. Control experiments in which neighboring centers were stimulated did not induce change. An explanation of the mechanisms involved is not yet available. (Supported by a grant from USPHS and Nat. Mul. Sc. Soc.)

7 *Physiology*

SPONTANEOUS AND EVOKED ACTIVITY OF SINGLE UNITS IN VISUAL CORTEX OF CAT DURING SLEEP AND WAKING. Edward V. Evarts, Natl. Insts. of Health, Bethesda, Md.

Recent studies have shown that gross cortical responses to lateral geniculate radiation stimulation are larger during sleep than during waking (Fed. Proc. 18:179, 1959). The present report is concerned with patterns of unit discharge underlying this phenomenon. Unit activity was recorded with tungsten microelectrodes, using a method modified after that of Hubel (J. Physiol. 147:226, 1959). Macroelectrodes were implanted for stimulating geniculate radiations and recording EEG. Observations were made with cats in darkness. Probability of discharge evoked in postsynaptic cortical units by geniculate radiation stimulation was reduced during waking as compared to sleep. In association with this reduction of evoked unit activity, there was also reduction of spontaneous discharge, sometimes to the point of complete arrest. The correlation between reduction of evoked discharge and reduction of spontaneous discharge during waking was +.51 ($p < .05$). These findings show that reduction of evoked discharge in these neurons during waking is not secondary to increased background activity. The results suggest, rather, that waking is associated with an increase of tonic inhibitory input to certain neurons in the visual cortex.

8 *Physiology*

FIRING LEVEL AND PREPOTENTIALS IN HIPPOCAMPAL NEURONS. W.A. Spencer and E.R. Kandel (intr. by C. Spyropoulos) N.I.H., Bethesda, Maryland.

Utilizing intracellular recordings for the study of hippocampal neurons in anesthetized cats, firing levels were measured for spikes occurring spontaneously, and for those initiated by orthodromic volleys and by intracellular stimulation. Major findings were: (1) In most units, firing level was nearly constant for the different modes of activation. (2) Critical threshold depolarization occurred (a) by slow graded prepotentials or (b) by fast prepotentials (F.P.P.s). The latter had all-or-none characteristics. The following observations suggest that the F.P.P.s represent spike activity generated within some part of the impaled neuron: (1) They appeared as a rebound to intracellularly delivered hyperpolarizing currents in "normal" cells. (2) They fired at increased frequencies when cells showed signs of injury. (3) When seen in isolation, the time constant of their falling phase was briefer than that of the passive membrane as measured by strength-latency and bridge unbalance. The small amplitude of the F.P.P.s (approx. 10% of spike) suggests an origin remote from the soma. Since F.P.P.s were never seen during antidromic activation, they probably do not arise in the axon. Therefore, it is suggested that they may arise somewhere in the elaborate dendritic tree of these cells.

9 *Physiology*

REPETITIVE FIRING OF HIPPOCAMPAL NEURONS. E.R. Kandel and W.A. Spencer (intr. by W.H. Marshall) N.I.H., Bethesda, Md.

Repetitive firing (bursts), generated spontaneously or evoked by stimulation through the intracellular recording microelectrode, was studied in hippocampal neurons of anesthetized cats. (1) Action potentials were followed by depolarizing afterpotentials (D.A.P.s) the decay time constant of which was independent of the number of spikes in the burst. D.A.P.s were additive; the membrane never hyperpolarized, but remained depolarized between spikes of a burst. Despite a progressive increase in depolarization of the B area, the frequency of firing, height of B spike and safety factor for AB invasion decreased with duration of the burst. The membrane repolarized following a burst even in the presence of long depolarizing currents, suggesting that the burst is self-limiting. These findings are compatible with the interpretation that refractoriness and inactivation of the spike generator limit frequency and duration of repetitive firing. (2) Bursts were produced as rebound to hyperpolarizing pulses and at times, continued after the "off" of depolarizing pulses. This occurrence of repetitive firing in the absence of applied current appears to represent an intraneuronal maintenance of an excitatory state and suggests that, once appropriately triggered, the soma membrane is capable of sustaining repetitive firing by summation of D.A.P.s.

10 *Physiology*

USE OF "MEMOSCOPE" FOR STUDY OF EVOKED POTENTIALS OF HUMAN EEG. James Toman and B. Weiss. Chicago Medical School, Chi.

Methodology is described for demonstration and averaging of low-level stimulus-evoked, subject-evoked or autocorrelated EEG potentials. EEG signal after preliminary amplification is electronically chopped at 1000 cycles, further amplified, and used to modulate beam intensity of an infinite-persistence cathode-ray oscilloscope (Hughes Aircraft "Memoscope"). Trace is triggered by desired stimulus or autocorrelation signal. Successive traces are shifted vertically by a clock mechanism. Accumulated responses appear as vertical stripes, while uncorrelated waves are randomly dispersed. Average conventional trace response is recorded by sweeping pattern horizontally with slit-entry photocell. Results include demonstration of attention factor in evoked auditory or visual responses.

/1 *Physiology*

LOCALIZATION OF SEPARATE CORTICAL AREAS FOR TASTE AND TACTILE TONGUE AFFERENTS IN SQUIRREL MONKEY (Saimiri sciureus). Robert M. Benjamin and Raimond Immers*. Dept. Physiol., Med. School, Univ. Wis., Madison.

Electrical stimulation of the chorda tympani produced evoked potentials in two separate areas of the cortex. One was located in the center of the evoked potential area for tactile afferents from the tongue; response latencies were comparable to those for tactile responses in the same area. It was concluded that these responses were due to the tactile fiber component of the chorda tympani. A second focus was found more rostrally in cortex not activated by tactile stimulation. Latencies for these responses were longer. It was concluded that these responses resulted from activation of taste fibers in the chorda tympani and that this was the true cortical receptive area for taste. These data suggest that the single chorda tympani area previously found in the cat's tactile tongue area probably is the homologue of the first or tactile area obtained in the monkey, and that the true taste area in the cat should be hidden in the presylvian fissure. (Supported by NIMDB, B-1932).

/2 *Physiology*

COMPARISON OF NEMBUTAL AND PROCAINE EFFECTS ON DIRECT CORTICAL RESPONSES IN ISOLATED CAT CORTEX. Michael J. Jerve,* Thomas G. Holmes,* Sidney Goldring and James L. O'Leary.* Washington Univ. School of Med., St. Louis, Mo.

Effects of Nembutal (30-60 mg. i.v. or 5% surface application) and Procaine (30-9- mg. i.v. or 5% surface application) on the direct cortical response (d.c.r.) were studied in isolated cortex (cat 1.5-2 kg.). All components of the non-isolated d.c.r. can be identified in isolated cortex; initial 15 msec. neg. potential, pos. after-effect and slow negativity. However, isolated d.c.r. has a lower threshold and larger pos. after-effect. Nembutal increases both pos. after-effect and slow negativity. Procaine diminishes them. In concentrations necessary to produce the above changes Nembutal reduces the initial neg. potential, Procaine leaves it unchanged. Following Nembutal, but not Procaine, and using strong stimuli the initial neg. potential is replaced by a pos. one. Then it fuses with the pos. after-effect to give a large pos. potential, resembling the change produced by gamma amino butyric acid. All above changes occur with either i.v. injection or surface application of the anesthetics. Thus Nembutal and Procaine affect d.c.r. components differently, the results showing the usefulness of d.c.r. for comparing drug effects on cerebral cortex.

/3 *Physiology*

DESCENDING CONNECTIONS OF AUDITORY CORTEX AND THALAMUS OF THE CAT. Jost B. Walther and Grant L. Rasmussen (intr. by W. F. Windle). NINDB, National Institutes of Health, Bethesda, Md.

The purpose of this study was to determine the corticogeniculate connections of the different auditory areas. Isolated lesions were placed in the cortex and antegrade axis-cylinder degeneration resulting after 6 days was traced in Nauta-stained sections. The principal part of the corpus geniculatum mediale (cgm) receives descending connections from AI, AII, the posterior ectosylvian area (Ep) and the insular and temporal regions, although apparently no fibers reach its dorsal posterior portion. Additional fibers descend to the magnocellular part of the cgm from at least AI, AII and Ep. The pattern of the descending connections resembles in general that of the corresponding ascending paths. Fibers which terminate in lower auditory centers are not considered here. The fact that Ep and the insular and temporal regions send descending connections to the cgm would lend further support to the more recent physiological studies which include these among the auditory areas. Since functional evidence is still lacking, it can only be assumed that the general concepts of the role of corticifugal systems may also apply to the connections described here. (Supported in part by the I.C.A., through the National Academy of Sciences.)

/4 *Physiology*

Cerebral Electrical Correlates of Optokinetic Stimulation. Stefan Shanzer,* Irving H. Wagman, Morris B. Bender, and Howard P. Krieger, Dept. of Neurol. Mt. Sinai Hospital, N.Y.C.

Electrical activity in the brain and brainstem of 18 monkeys was recorded through implanted electrodes during optokinetic stimulation inside a drum filling the field of vision. Eye movements were recorded simultaneously. Only in both calcarine regions did the passing stripes evoke specific electrical activity. This consisted of sharp waves and spikes correlated with the rate of passage of stripes but not necessarily with the oculomotor response. During normally occurring afternystagmus in darkness this synchronous activity was absent. Calorically induced nystagmus produced comparable calcarine activity if recording was done in an illuminated room. If recording was done in the dark, the synchronous calcarine activity disappeared but the nystagmus was either unaffected or augmented. In monkeys with paralysis of gaze due to pontine lesions, optokinetic stimulation failed to induce nystagmus although it evoked calcarine electrical activity indistinguishable from the normal. These observations indicate that the evoked calcarine activity correlates with visual input rather than oculomotor output. (Supported by USPHS Grants Nos. B-294 and B-1040.)

/5 *Physiology*

THALAMOCORTICAL RECRUITING RESPONSES AND RELAYED DISCHARGES IN PYRAMIDAL TRACT. D. P. Purpura and E. M. Housepian*, Dept. of Neurosurg., College of Physicians and Surgeons, New York.

Relayed pyramidal tract discharges (RPTD's) associated with augmenting responses and those accompanying 'short-latency' recruiting responses (r. r. 's) to n. AM, VA and Ret. stimulation in locally anesthetized-paralyzed cats are identified as independent but closely related events. R. R. 's with prominent initial positivity are associated with 8-15 msec latency 'recruiting' RPTD's whereas r. r. 's with minimal or absent initial surface positivity are correlated 20-30msec latency RPTD's. Both varieties of r.r.'s are maximal in pre-cruciate and suprasylvian cortex, show little recruitment and fail to "wax and wane" prior to nembutal administration (4-10 mg/K). During 8-12/sec thalamic stimulation inhibition of tested direct and indirect tract discharges evoked by motor cortex stimulation is detectable prior to RPTD's, but this is converted to facilitation with 25-30/sec stimulation. Inhibition of 2 msec latency RPTD's associated with non-augmenting responses to VPL and VL stimulation also occurs prior to the development of overt surface-potential changes.

/6 *Pharmacology*

MODIFICATION OF THE CENTRAL EFFECT OF MORPHINE BY QUATERNARY AMMONIUM COMPOUNDS. Leonide Goldstein (intr. by Carl C. Pfeiffer). Dept. of Pharmacology, Emory University, Atlanta 22, Georgia.

It has been reported by Szerb (Arch. Int. Pharm. 61:314, 1957) that neostigmine potentiates the analgesic effect of morphine. It has now been found that the threshold for the depressant action of morphine on the EEG in unanesthetized, unrestrained rabbits with permanent electrodes is lowered by the previous administration of certain quaternary ammonium compounds which are devoid, at the dose used, of pharmacological action. Under the same conditions, tertiary compounds do not enhance the morphine effect. Drugs such as neostigmine, methyl-atropine, methyl-scopolamine, d-tubocurarine and choline lower the threshold for the effect of morphine on the EEG, while drugs such as physostigmine, atropine, scopolamine and deanol do not. The effect is obtained with cholinergic stimulants as well as with anti-cholinergic drugs. Adrenergic blockade does not sensitize the typical morphine effect. These results will be discussed and a comparison will be made of the interaction of the compounds with morphine and nalorphine.

XUM

17 *Immunology*

INTER- AND INTRA-HEMISPHERIC TRANSMISSION OF TACTILE GNOSIS IN NORMAL AND CORPUS CALLOSUM-SECTIONED MONKEYS. F. Francis Ebner and Ronald E. Myers (intr. by Richard P. Mason.) Walter Reed Army Institute of Research, Washington, D.C.

Normal monkeys recognize immediately with one hand tactile discriminations learned with the other hand. They fail to recognize immediately, however, on tests with the feet but the prior hand experience greatly facilitates subsequent relearning. After one foot achieves complete recognition of the discriminanda, the second foot has no immediate insight but evidences great saving in relearning trials. Final level of performance achieved with the feet usually equals that attained with the hands. Section of the corpus callosum prior to training in naive animals completely disrupted cross-recognition between the hands as well as between the feet.

190. NEUROPHYSIOLOGY–Brain–Hypothalamus

1 *Physiology*

MEDIAN FOREBRAIN BUNDLE AND HYPOTHALAMIC "FEEDING" CENTERS. P. J. Morgane (Intr. by S. R. Bruesch). Department of Physiology, University of Tennessee Medical Units, Memphis, Tenn.

Experimental studies indicate that the limbic system is primarily concerned with neural organization of feeding behavior. This primitive system feeds into the hypothalamic nuclei via the median forebrain bundle (M.F.B.). Recent evidence has suggested that this bundle may be as important as the lateral hypothalamic area proper in the control of food intake (Am. J. Physiol. 193/230, 1958). This hypothesis was tested by placing stereotaxic lesions in the M.F.B. of rats at various diencephalic levels. Lesions in this system rostral or caudal to the coronal plane of the median eminence did not significantly alter the food intake or body weights from control values while lesions at the tuberal level resulted in consistently reproducible aphagia and loss of body weight. Duration of the aphagic period was variable. Current flows of 2mA. for 15 sec. produced permanent aphagia and death of the animal between 10 and 12 days. 1 mA. for 15 sec. was associated with aphagic "escape" (aphagia with spontaneous recovery) between 5 and 7 days. Sham operations (electrodes inserted at same coordinates with no current flow) resulted in 3-5 day aphagic periods and subsequent "escape". It is suggested that the lateral hypothalamic neurons themselves at the mid-hypothalamic level are the critical components in normal function of the "feeding" center.

2 *Physiology*

INHIBITION OF ADRENAL CORTICOID RELEASE IN BRAINSTEM-STIMULATED CATS. Margaret A. Slusher* and Jane E. Hyde. Depts. Anatomy, Physiol. Chem., and Physiology, UCLA Med. Ctr. Los Angeles.

Four hours after high cervical section (under ether), 30 sec. stimulation of midbrain or posterior diencephalic sites in 17 male cats resulted in alteration in adrenal corticoid effluent levels. Decreases from pre-stimulation levels of 21-67% (aver. 32%) in 11 cats, and increases of 24-125% (aver. 70%) in 6 cats were noted. Alterations after stimulation (3 volts, 25/sec.) were significantly greater than changes noted in 4 non-stimulated cats. 2 hours later the original diencephalic site in 5 cats was stimulated simultaneously with a midbrain site; the corticoid picture was the reverse of that noted following single stimulation. Adrenal venous effluent samples were analyzed by chromatographic and colorimetric methods from total 3-10 min. collections obtained periodically during 8-12 hours after cord section. Condition of cat was monitored by blood pressure, temperature, ECG, spontaneous eye movements and pupil size. Blood volume lost by effluent collection was replaced by i.v. drip of cat blood. Results indicate interaction between inhibitory and stimulatory components in both diencephalic and midbrain regions for control of ACTH release.

3 *Physiology*

ISOLATED BRAIN AND ACTH RELEASE. E. S. Redgate (intr. by R. W. Eckstein). Dept. of Physiol., Western Reserve Univ., Cleveland, Ohio.

The destination of an ascending spinal pathway mediating ACTH release has been localized in a region rostral to the pons by the following series of experiments. Electrical stimulation of the hypothalamus of the brain isolated by pretrigeminal transection in adrenalectomized rats resulted in ACTH release; whereas, electrical stimulation of afferent paths caudal to the transection which has been shown to mediate ACTH release in intact rats, does not result in ACTH release. The ACTH content of blood samples from 3 groups of isolated brain rats was assayed in recipient hypophysectomized rats by the adrenal ascorbic acid depletion method (AAAD). Three consecutive jugular vein blood samples from each of a group of 24 donor rats induced the following AAAD (mean ± S.E.) #1, 35 ± 7 (24); #2, 45 ± 9 (24); #3, 34 ± 11 (14). Three consecutive blood samples were taken from another group of 24 rats which were electrically stimulated through a hypothalamic electrode during withdrawal of blood sample #2. The AAAD's were: #1, 48 ± 8 (24); #2, during stimulation, 102 ± 9 (24); #3, 48 ± 12 (8). Consequently, it is now possible to state that the ACTH releasing mechanism which is activated by afferent tract discharge lies rostral to the pons.

4 *Physiology*

ASCORBIC ACID CONTENT OF ENDOCRINE GLANDS IN PERSISTENT ESTRUS HYPOTHALAMIC LESIONED RATS. Savino A. D'Angelo and Joseph Snyder*. Jefferson Medical College, Philadelphia, Pa.

Ascorbic acid content of the adenohypophysis, ovary and adrenal were determined (Roe-Keuther) in female rats 4-10 months after persistent estrus had been induced by placement of bilateral electrolytic lesions in pre-optic and anterior hypothalamic brain areas. Ascorbic acid content of the pituitary significantly increased in lesioned rats, paralleling gland enlargement. Uterine hypertrophy occurred with decreased ovarian weight but ascorbic acid concentration in the gonad remained normal. Weight and ascorbic acid concentration of the adrenal in lesioned animals were not significantly altered. Exposure of operated rats to low temperature (5°C) for 2-7 weeks before sacrifice profoundly altered pituitary-gonadal and adrenal function. The estrous vaginal smear was replaced by one of sustained diestrus. There was uterine involution and pronounced atrophy of the ovary; ascorbic acid concentration in the latter was normal. Adrenal enlargement with increased ascorbic acid content resulted from cold exposure. Pituitary ascorbic acid decreased in refrigerated animals. The results demonstrate that marked shifts in hypothalamo-hypophysial-target gland function are not reflected by ascorbic acid concentration in the endocrine glands. (supported by grant A-432, National Institutes of Health, PHS.

5 *Physiology*

STUDIES ON NEURAL REGULATION OF LH. S.M.McCann, S.Taleisnik*, and H. Friedman*, Dept. of Physiol., U. of Penn., Phila., Pa.

The ovarian ascorbic acid depletion test of Parlow (Fed. Proc. 17:402, 1958) has been used as an assay for measuring LH in immature or adult female rats. This test is sensitive, reasonably precise and highly specific for LH as judged by the response to LH as compared to FSH, prolactin, or ACTH. Various stimuli such as surgical trauma, hemorrhage, epinephrine, and nicotine had little or no effect on ovarian ascorbic acid. Vasopressin, but not oxytocin, had LH-like activity in pharmacological doses. One week following ovariectomy, significant titers of LH were found in plasma (on assay in immature animals), and these elevated levels were maintained for at least 6 weeks. Lesions in the median eminence, or hypothalamic damage associated with a state of persistent vaginal estrus prevented this elevation in LH after ovariectomy. Three weeks post-median eminence lesions, pituitary LH was within normal limits, but it was markedly decreased several months post-lesions. The mere passage of an electrode into the hypothalamus of the adult rat induced ovarian ascorbic acid depletion. The results give further evidence for hypothalamic control over LH release.

6 *Physiology*

MIDBRAIN LESIONS AND ALDOSTERONE. A.Newman Taylor (intr. by J. M. Werle). Physiol. Dept.,Western Reserve Univ.,Cleveland, O.

Earlier studies indicated that the midbrain and caudal diencephalon contain both stimulatory and inhibitory components of the aldosterone regulatory mechanism (Endocrinol.63:723,1958). Small brain stem lesions (high frequency coagulation) were made in 14 male cats deprived of sodium 2 to 3 weeks. Four hours after placing the lesion, adrenal venous blood was collected for paper chromatographic isolation of aldosterone. All lesions destroyed posterior commissure, central gray substance and structures immediately lateral to it in the rostral midbrain, and either the subcommissural organ or pineal body, or both. Aldosterone output in 9 control cats without lesions was 65.8±4.8 (S.E.) µg/100 kg.body wt./hr. The χ^2 distribution of the aldosterone levels in the group with lesions was significantly different from that of the controls ($P<.01$). Complete destruction of the subcommissural area without pineal damage resulted in lowest levels of aldosterone (16.04, 30.77). A lesion sparing the subcommissural area but damaging the pineal gave elevated output (122.40). When both the subcommissural area and pineal were damaged, levels were: 44.34, 45.60, 46.37, 46.90, 47.69, 47.70, 48.05, 59.68, 61.81, 62.51, 71.46. These findings suggest that the subcommissural area may be a locus of stimulation, either humoral or neuronal, whereas the pineal may be the source of inhibitory influences.

7 *Physiology*

OSMOTIC ACTIVATION OF THE NEUROHYPOPHYSIAL MILK-EJECTION REFLEX IN RABBITS WITH "DIENCEPHALIC ISLANDS". John W. Sundsten* & Charles H. Sawyer. Sch. of Med., Univ. of Calif., Los Angeles.

Intracarotid injections of hypertonic solutions have been used in attempts to localize osmosensitive elements within the brain. Such injections cause electroencephalographic (EEG) changes that emanate from the olfactory bulb (Proc. Soc. Exptl. Biol. & Med. 101:524, 1959) as well as the release of antidiuretic hormone and oxytocin from the neurohypophysis. To determine whether the EEG changes are causally related to neurohypophysial activation, the effects of forebrain ablations on the responses were studied. The release of oxytocin was assessed by the milk-ejection technique (Am. J. Physiol. 196: 791, 1959). In 9 locally anesthetized immobilized lactating rabbits, removal of the olfactory bulbs did not eliminate the milk-ejection response to hypertonic saline. In 4 of these acute preparations, "diencephalic islands" were produced by transecting at the rostral and caudal limits of the hypothalamus and sectioning parasagittally approximately 1 cm from the midline. Subsequently, the osmotic stimulus was still able to induce a milk-ejection response of undiminished amplitude. It would appear that whatever role osmosensitive elements in the rest of the brain may play, there are, as Verney proposed, receptors in the diencephalon capable of activating the neurohypophysis in response to hyperosmotic stimuli.

8 *Physiology*

SPREADING DEPRESSION AND SELF-STIMULATION. James Olds and Robert P. Travis.* Univ. of Mich., Ann Arbor.

The effect of spreading cortical depression (SD) on hypothalamic self-stimulation and on tegmental escape responses was studied in unanesthetized rats. SD was evoked by 22% KCl applied through trephine openings following the procedure of Bureš. This method has been shown to cause repeated waves of SD lasting 3 to 5 hours. Animals were trained to an 8-minute cycle. During the first 2-minute interval hypothalamic stimulation was available and all animals self-stimulated. During the second 2-minute interval tegmental stimulation was available but none of the animals self-stimulated. During the third interval, hypothalamic stimulation was delivered by a programmer and interrupted for 4 seconds by the animal's response. Depending on electrode locus, some animals responded regularly to terminate the stimulus, others failed to escape from the hypothalamic stimulus. During the fourth interval, tegmental stimulation was similarly delivered, and all animals responded regularly to escape it. Against this background, SD caused an almost immediate cessation of self-stimulation. During the second hour, tegmental escape behavior was depressed and sometimes abolished. During the third hour escape behavior returned. By the end of the fourth hour, self-stimulation behavior returned. This set of changes could also be evoked in the same animals by a 2 mg/kg injection of chlorpromazine.

9 *Biochemistry*

γ-BUTYROBETAINE ESTERS ON AMMONIA METABOLISM. E.A.Hosein (intr.by O.F.Denstedt) Dept. of Biochemistry, McGill University, Montreal, P.Q.

Injection of cerebral convulsants into rats causes release in brain of γ-butyrobetaine (gBBE),crotonbetaine,carnitine and their coenzyme A esters. Betaine esters have ACh-like properties,thus these CoA esters might be transmitter substances. We have used methyl (gBBM),ethyl (gBBE) and choline (gBBC) esters of gBB as models of the betaine ester which, released in vivo, could initiate convulsions. During convulsions, the ammonia content of brain is increased.Incubation of rat cerebral cortex slices with 1 mM gBB esters caused increased ammonia release. Synthesis of glutamic acid (GA) from ammonia and α-ketoglutaric is inhibited ;50% by 1 mM gBB esters. Glutamine synthesis from ammonia and GA was similarly inhibited. Brain glutaminase was unaffected by 0.3-10 mM gBBM. Incubating brain slices with either β-alanine, GA or GABA and gBBC resulted in stimulation (20-40%) of ammonia formed. During convulsions,brain ammonia could increase through deamination of many substrates and by betaine ester inhibition of certain enzyme systems requiring ammonia. Supported by grant from USPH, Project B-1756.

10 *Pathology*

AGGRESSIVE BEHAVIOR PRODUCED BY ELECTRICAL STIMULATION OF THE VENTROMEDIAL NUCLEUS OF THE HYPOTHALAMUS IN THE CAT. Murray Glusman and Leon Roizin (intr. by A. Weil.), Depts. of Experimental Psychiatry and Neuropathology. N.Y. State Psychiatric Institute and Dept. of Psychiatry, Columbia Univ.

In a series of cats with electrodes chronically implanted in the hypothalamus, electrical stimulation produced a variety of prominent behavioral responses. Histological verification of electrode placements in these animals indicates that unilateral stimulation of the ventromedial nucleus of the hypothalamus (HVM) or its immediate vicinity can produce typical "rage" behavior culminating in well directed attack. Since Wheatley (Arch. Neurol. and Psychiat., Chi. 52: 296, 1944) demonstrated that bilateral destruction of HVM in the cat produces permanent savage behavior, it is evident that aggressive behavior can be produced either by stimulating or destroying HVM. The paradoxically similar effects of stimulation and destruction may be explained by assuming that the electrical stimulus temporarily halts function while the lesion permanently interrupts function. Alternatively, it may be hypothesized that bilateral destruction of HVM produces savage behavior by creating a state of "denervation supersensitivity" in brain stem elements involved in the mediation of rage.

191. NEUROPHYSIOLOGY–Brain–Mid-brain

1 *Pharmacology*

A CORTICAL RECRUITING RESPONSE ELICITED BY LOW-FREQUENCY STIMULATION OF THE MESENCEPHALIC RETICULAR FORMATION. T.E. Lynes (intr. by J.A. Bain) Emory University, Atlanta, Ga.

In 1958 O'Leary and coworkers described a cortical recruiting response which could be elicited by low frequency stimulation of the rostral pole of the mesencephalic reticular formation in the rabbit. The present study was undertaken to determine whether a similar response could be elicited from this area in the cat and, in addition, to attempt to produce reticular activation as well from a single stimulating electrode. Bipolar stimulating electrodes were placed in the rostral mesencephalic reticular formation at Horsley-Clarke coordinates A2, L3, H-1-3 in both chronic and acute cats. Stimulation in this area at a frequency of 8 cycles/sec produced a long latency (40 msec), widely distributed cortical response which waxed and waned during the duration of the stimulus. Stimulation at 300 cycles/sec in the same area produced the usual cortical arousal response. Low doses of pentobarbital lowered, whereas mephenesin increased the threshold for the production of this response. The results indicate that the response of the anterior pole of the mesencephalic reticular formation to low frequency stimulation in these studies can be termed a "true recruiting response" and that both recruiting and arousal responses can be obtained from the same area of the brain stem.

XUM

192. NEUROPHYSIOLOGY–
Catechol Amines

1 **Physiology**

AN UNIDENTIFIED ACIDIC CATABOLITE OF DL-NORADRENALINE IN THE HUMAN. Lawrence Rosen* and McC. Goodall. Memorial Research Center and Hospital, Univ. of Tenn., Knoxville, Tenn.

Six healthy males (20-35 years) were infused with 9.5μc of dl-noradrenaline-2C^{14} (specific activity, 20 mc/mmole). Following infusion, the urines were collected for 24 hours and the radioactive catabolites were fractionated according to the procedure of Goodall, Kirshner, and Rosen (J. Clin. Invest., 1959, 38, 707). Among the acidic catabolites isolated was one which exhibited R_f values of 0.2 in isopropanol-ammonia and 0.3 in n-butanol-hydrochloric acid. This compound accounted for approximately 10% of the urinary radioactivity. It could not be extracted into ether from either acidic or basic solution. After heating this compound at 100° for 60 or 120 minutes in 3 N hydrochloric acid, two fast moving chromatographic peaks were observed in n-butanol-hydrochloric acid. These peaks were extractable into ether from acidic solution. Bacterial (Sigma) or animal (Warner-Chilcott) glucuronidase as well as mild conditions of acid hydrolysis were without apparent effect on this compound. (Supported by U.S.P.H.S. Grant H-4311)

2 **Physiology**

COMPARISON OF CHEMICAL AND BIOLOGICAL DETERMINATION OF BRAIN CATECHOL AMINES. G.R. Pscheidt and Clara Morpurgo (intr. by H.E. Himwich). State Research Hospital, Galesburg, Illinois.

Comparable increases of brain catechol amines were obtained in rats and rabbits given monoamine oxidase inhibitors using either a chemical (trihydroxyindole) or biological (cat blood pressure) method. The hypertensive activity of midbrain extracts prepared from dogs given single injections of either phenyl isopropyl hydrazine (JB 516, 10 mg/kg i.m.) or tranylcypromine (SKF 385, 2 mg/kg i.v.) was increased several fold over normal levels. This increase paralleled a corresponding rise in the serotonin content of the midbrain. However, the catechol amine values obtained by the chemical method were not much above normal in JB 516 treated dogs. Results of additional experiments designed to resolve the discrepancy between the two assay procedures as applied to dog brain will be presented.

3 **Physiology**

FORMATION AND METABOLISM OF N-ACETYLNORMETANEPHRINE IN RATS TREATED WITH IPRONIAZID. Alfred Smith* and S.Bernard Wortis. Dept. of Psychiatry, New York Univ. College of Medicine, N.Y.

Alternate pathways for the metabolism of amines may become more apparent following inhibition of monoamine oxidase. This possibility was investigated using normetanephrine-1-C^{14}. After treatment with 100 mg/kg ipropiazid, 3 white rats were each given 6 ug normetanephrine-1-C^{14} and their urines collected during an 8 hour period. Similar experiments were performed with untreated rats. Chromatograms were prepared from the whole urines incubated with glucuronidase. Detected only in the urines of iproniazid-treated rats was a compound having the same Rf's in several solvents as N-acetylnormetanephrine. This substance was further characterized by its adsorption properties and by its conversion to normetanephrine following acid hydrolysis. The metabolism of this compound was then studied in iproniazid-treated rats. Following injection this compound was largely excreted as the glucuronide and a smaller amount appeared to be sulfated. Adsorption studies failed, to reveal basic nitrogen which suggests the irreversibility of of N-acetylation under these conditions. This study has shown that normetanephrine is N-acetylated in the iproniazid-treated rat and this compound is excreted in the urine largely as the glucuronide. (Supported by a grant M-1434(C3) from the United States Public Health Service)

4 **Physiology**

METABOLISM OF DL-ADRENALINE-2-C^{14} IN THE CAT. Norman Kirshner, Lewis Terry and D. D. Pollard. (intr. by K. S. Grimson), Dept. of Biochemistry, Duke Univ. Durham, N. C.

Studies on the metabolism of adrenaline (A) in the cat have been undertaken to determine the roles played by the various tissues and organs in the formation of A catabolites. Initial studies of the urinary catabolites show the major products to be 3-O-methyladrenaline (MA), 3-methoxy-4-hydroxymandelic acid (MOMA), a conjugate of MOMA, and a conjugate of a neutral compound. After pretreatment of the cats with pyrogallol(Science, 130, 453, (1959)) the major products are 3,4-dihydroxymandelic acid (DOMA), a compound which appears to be a conjugate of DOMA, and a neutral compound which differs from the neutral compound formed in untreated cats. The conjugates are not hydrolysed by B-glucuronidase but are cleaved by Mylase P (Sigma). Following intravenous infusions of A-C^{14}, the distribution of radioactivity, of A-C^{14} and its metabolic products in liver, heart, spleen, lung, kidney, viscera, blood, brain, diaphragm and skeletal muscle were determined by a combination of filter paper and ion exchange chromatography. (J. Pharm. Expt'l Therap. 127, 1, (1959)). Within five minutes after the infusion only trace amounts of A-C^{14} can be found; MA and one or more neutral compounds account for most of the recovered radioactivity.

5 **Physiology**

DETERMINATION OF URINARY 3-METHOXY-4-HYDROXYMANDELIC ACID IN MAN. V.K. Weise*, R.K. McDonald, and E.H. LaBrosse*. Natl. Insts. of Health, Bethesda, Md.

3-Methoxy-4-hydroxymandelic acid (VMA) has been shown to be a major metabolite of both epinephrine and norepinephrine. In man, 30-40% of infused H^3-epinephrine and norepinephrine appears in the urine as H^3-VMA. A method for the determination of urinary VMA has been developed. A tracer amount of tritiated VMA is added to the urine to be assayed; the mixture is acidified, extracted with ethyl acetate and flash-evaporated. An aqueous solution of this material is loaded on a Dowex 1 (acetate form) ion-exchange column. Partial separation of the phenolic acids is effected by elution with 1 M formic acid adjusted to pH 8.0 with ammonium hydroxide. VMA is the first phenolic acid to appear in the eluate and, in some cases, is quantitatively separated from the other phenolic acids. However, certain other phenolic acids which fluctuate widely in concentration, as a result of dietary influences, will partially overlap the VMA when present in large amounts. A pool of those fractions containing only VMA is assayed (1) colorimetrically using diazotized p-nitroaniline, and (2) radioactively for tritium. Normal values and diurnal variation of VMA excretion will be presented.

6 **Physiology**

Thyroxin and Reserpine on Rate of Atrial Contraction. David Thier and J. S. Gravenstein (Intr. by E. R. Woodward.) Div. of Anesth., Coll. of Med., Univ. of Fla., Gainesville, Fla.

Isolated atria of hyperthyroid rats beat faster than atria of euthyroid rats. To test whether catechol amines present in the atria significantly contribute to the effect of thyroxin on atrial rate of contraction, 5 groups of 10 male rats each were prepared as follows: group 1, untreated; group 2, thyroxin 1.0 mg i.p. for 3 days; group 3, reserpine 5.0 mg/kg i.p. for 2 days; group 4, thyroxin followed by reserpine; group 5, reserpine followed by thyroxin. Groups 4 and 5 received the dosages of groups 2 and 3. One day after the last dose, the excised atria were suspended in an oxygenated bath, and their rate of contraction plotted against temperatures from 28°C to 41°C. 5 curves were obtained, each representing the mean of rate values of one experimental group. All curves showed low rates in the low temperature range and high rates at the higher temperature. While similar in shape, the curves were different in position. The atria from group 3 (reserpinized) beat slowest, those from group 2 (thyroxin) fastest. The control group was intermediate. Groups 4 and 5 were indistinguishable from each other and situated closely with the thyroxin group. Thus, in this preparation, thyroxin either affects rate independently of catechol amines, or it interferes with the action of reserpine.

7 **Biochemistry**

CONVERSION OF DOPAMINE TO NOREPINEPHRINE AND TO 3-METHOXYNOREPINEPHRINE IN ADRENOLECTOMIZED RATS. A.J. Friedhoff, M.Goldstein, C.Simmons and S.B.Wortis (intr. by Eric J. Simon). N.Y.U. College of Medicine, New York.

The conversion of dopamine to norepinephrine (NE), in vivo, which we have shown earlier (Fed.Proc.18:930,1959), has now been demonstrated to occur also in adrenolectomized rats. A group of normal and adrenolectomized rats was treated with iproniazid (100 mg/kg) and 100 ug dopamine-1-C^{14} (DA) daily for 3 days. A 24 hr. urine specimen was collected from each group. NE and 3-methoxynorepinephrine (3-MNE) were isolated from the urine by procedures described earlier (Proc. Exp. Biol. and Med., in press). In the urine of adrenolectomized rats collected during the first 24 hrs. of treatment, the activity of NE was found to be 2300 cts/min, and that of 3-MNE 5600 cts/min, whereas in urine of normal rats the activity of NE was found to be only 620 cts/min, and that of 3-MNE 1250 cts/min. In urine obtained from adrenolectomized rats during the third day of treatment, the activity of NE was found to be 3075 cts/min and that of 3-MNE 10,000 cts/min. In the third day specimen obtained from normal rats, the activity of NE was found to be 2000 cts/min and that of 3-MNE 4400 cts/min. The higher urinary excretion of NE-C^{14} and 3-MNE-C^{14} in adrenolectomized rats suggests that either the storage of NE is impaired or the synthesis is enhanced by adrenolectomy.

8 **Biochemistry**

EFFECTS OF CATECHOLAMINO ACIDS ON CATECHOLAMINE (CA) CONTENT OF RAT ORGANS. T. L. Sourkes and G. F. Murphy.* Allan Memorial Institute, McGill Univ., Montreal, Canada.

CAs, L-DP (3,4-dopa) and DL-AMDP (α-methylDP) were measured in organ extracts by differential spectrophotofluorometry (Rev. Can. de Biol. in press 1959). Injection of DP (20 mg/kg i.v.) into iproniazid-treated rats led to increased dopamine content of kidneys and brain, and appearance of this CA in heart, but not liver. DP was not detectable in kidney extracts by fluorometric means. By paper chromatography (n-butanol acetic acid) dopamine (Rf=0.40) and a faster component (Rf=0.51) were detected. AMDP (100 mg/kg i.p.) penetrated into brain, kidney, liver and spleen. Concentrations were relatively constant in the first two organs for at least 90 min. but were highest in the others immediately after injection, thereafter declining steadily for 2.5 hr. AMDP diminished brain dopamine (by 85%) and noradrenaline (30%) found one min. after i.v. injection of DP. Sufficient AMDP enters the brain to inhibit DP decarboxylase significantly (Arch. Biochem. Biophys. 51, 444, 1954). The following compounds given 0.5 hr. before DP (rats killed 6 min. after DP injection) affected brain dopamine as follows (% of control): AMDP (44); α-methyl-3-OH-4-OCH_3-phenylalanine (103); α-methyl-3-OH-phenylalanine (61); α-methyl-3-OCH_3-phenylalanine (115).

9 **Biochemistry**

IDENTIFICATION OF OCTOPAMINE IN ANIMALS TREATED WITH MONOAMINE OXIDASE INHIBITORS. Y. Kakimoto* and M. D. Armstrong. Fels Research Institute, Yellow Springs, Ohio.

p-Hydroxymandelic acid(HMA) is an endogenously produced metabolite;all specimens of human urine contain amounts usually in the range of .6 to 2.5 μg/mg. creatinine. Its identification is based upon R_F values, qualitative reactions on paper, and conversion by treatment with red P and HI of material eluted from chromatograms to a substance having the chromatographic properties of p-hydroxyphenylacetic acid. It seemed likely that HMA might be a metabolite of octopamine(OA). OA can be detected by absorption onto Dowex-50(H^+) followed by elution with ammoniacal EtOH and 2-dimensional paper chromatography of the eluate. OA could not be observed in urine of humans, rats, or rabbits or in extracts of tissues from rats and rabbits. However, OA can be detected in urine of rabbits treated with MAO inhibitors, Marsilid and Catron, and it becomes the most prominent phenolic amine in extracts of some tissues from these animals. Identification of OA is based upon comparison of authentic OA and material from Marsilid treated rabbit urine purified by ion-exchange chromatography as regards: UV adsorption spectrum, fluorescence spectrum, R_F values of free amine and N-acetylamine, color reactions on paper, and behavior upon chromatography on Amberlite IRC-50. (Supported in part by USPHS Research grant M-2278.)

10 **Biochemistry**

THE METABOLIC FATE OF EPINEPHRINE IN THE RAT. Irwin J. Kopin*, and Julius Axelrod. National Institutes of Health, Bethesda, Md.

The simultaneous administration of DL-epinephrine-7-H^3 and DL-metanephrine-methoxy-C^{14} allows estimation of the relative magnitude of O-methylation and deamination. Specific methods have been developed for the isolation and determination of the various metabolites. About 90% of the administered H^3 can be accounted for in the urine and feces. Less C^{14} (75%) is found because of O-methylation. The major methylated metabolites of epinephrine are metanephrine (10% free, 22% conjugated), 3-methoxy-4-hydroxyphenylglycol sulfate (20%), and vanillyl-mandelic acid (8%). From the H^3/C^{14} ratios in the metabolites it was estimated that about 70% of the epinephrine was O-methylated and direct deamination constituted a relatively minor pathway.

11 **Pharmacology**

THE DISTRIBUTION AND METABOLISM OF SIMULTANEOUSLY INFUSED NOREPINEPHRINE-7-H^3 AND EPINEPHRINE-1-C^{14}. M.Goldstein,* A.J. Friedhoff,* S.Pomerantz* and S.B. Gertner. N.Y.U. Schl. of Med., N.Y. and Seton Hall Col. of Med., Jersey City, N.J.

10 ml.of $4.5x10^{-6}$M solution of norepinephrine-7-H^3 (NE)and epinephrine-1-C^{14} (E) with a H^3:C^{14} ratio of 5:1 were infused for 30 min. into the femoral vein of untreated and iproniazid treated cats. By extraction with ethylacetate and adsorption on alumina, catechol amine (CA), methoxycatechol amine (MCA) and acidic (A) fractions were obtained from various organs.The H^3:C^{14} ratio of the CA fraction in the heart,liver and spleen was found to be increased compared to the original infused solution, whereas this ratio in the A and MCA fractions was decreased. In cats treated with iproniazid higher activities were found in the MCA and CA fractions. A H^3:C^{14} ratio of 2.5:1 was found for 3,4-dihydroxymandelic acid and 3:1 for 3-methoxy-4-hydroxymandelic acid,which were isolated from the A fraction of liver by paper chromatography. NE-H^3 and E-C^{14} were isolated from the CA fraction of spleen,heart,liver and adrenals. 3-methoxy NE-H^3 and 3-methoxy E-C^{14} were isolated from the MCA fraction of spleen,liver,heart and kidney. This data shows that E is more rapidly metabolized than NE. The isolation of E-H^3 and 3-methoxy E-H^3 from the liver, adrenals and kidney indicates that NE is N-methylated to E in these organs.

12 **Pharmacology**

A HYPERTENSIVE PHASE OF PHENOXYBENZAMINE ACTION. B. G. Benfey, G. Ledoux* and K. I. Melville. Department of Pharmacology, McGill University, Montreal, Canada.

In dogs under pentobarbital anesthesia, the i.v. injection of 20 mg./kg. phenoxybenzamine after ganglion-blocking drugs induced a progressive rise in blood pressure, followed by the usual depressor response. This appeared to be similar to the effect of injected reserpine or hexahydro-1-azepine-propionamidoxime after ganglion-blocking drugs (Maxwell et al., J. Pharmacol. 119, 69, 1957, and 123, 128, 1958). However, during the hypertensive phase, the injection of phentolamine led to a further brief increase in blood pressure. Sympathetic blockade by guanethidine inhibited the pressure rise after phenoxybenzamine and restored the depressor response to phentolamine. The hypertensive response seems to be related to the increased plasma concentration and urinary excretion of epinephrine and norepinephrine after phenoxybenzamine reported by us (Brit. J. Pharmacol. 14, 9 and 142, 1959). (Supported by a grant from the National Research Council of Canada.)

XUM

13

Pharmacology

BLOCKADE OF THE HYPERGLYCEMIC ACTION OF EPINEPHRINE BY DICHLOROISOPROTERENOL. S.E. Mayer and N.C. Moran. Dept. Pharmacology, Emory University, Atlanta, Ga.

Dichloroisoproterenol (DCI) prevents the activation of myocardial phosphorylase by epinephrine (E), as it blocks the positive inotropic action of E in the dog heart (Mayer & Moran, Fed. Proc. 18:419, 1959). Since phosphorylase activation probably plays a role in the hyperglycemic action of E (Sutherland, Rec. Prog. Horm. Res. 5:441, 1950), the effect of DCI on the stimulant action of E on carbohydrate metabolism was examined. In dogs 1 µg/kg/min of E infused i.v. for 15 min raised blood glucose levels an average of 60.6±S.E. 8.6 mg/100 ml and blood lactate 8.12±1.79 mg/100 ml. After 7 mg/kg of DCI the blood glucose response to epinephrine was reduced to 18.1±3.1 mg/100 ml and the blood lactate response to 2.78±0.97 mg/100 ml. DCI itself produced small and irregular changes in these carbohydrates. The blockade of the hyperglycemic action of E by DCI points to a similarity between the receptors for this metabolic action and the positive inotropic action of E, i.e. that both are beta by Ahlquist's classification.

14

Pharmacology

SOME INTERACTIONS BETWEEN PRESSOR PHENYLALKYLAMINES AND AGENTS WHICH PRODUCE A DIFFERENTIAL BLOCK OF PHENYLALKYLAMINES. R. A. Maxwell, F. Schneider,* H. Coombs,* H. Povalski* and A. J. Plummer. Research Dept., CIBA Pharmaceutical Products Inc., Summit, N. J.

The potentiating effect which cocaine exerts on the pressor actions of norepinephrine in the dog are not suppressed by pretreatment with reserpine. Cocaine can reverse the phentolamine-induced blockade of norepinephrine pressor responses in dogs which have been pretreated with reserpine. In dogs, two weeks after guanethidine treatment, when aorta catecholamine levels are still below normal the pressor responses to amphetamine are suppressed but the pressor responses to tyramine are not. In rats at 24 hours after pretreatment with guanethidine, when the heart levels of catecholamines have returned to normal the pressor actions of amphetamine and tyramine are clearly suppressed.

15

Pharmacology

A DIFFERENTIAL EFFECT OF GUANETHIDINE ON THE CARDIOVASCULAR ACTION OF PRESSOR PHENYLALKYLAMINES IN THE DOG. H. Povalski,* R. Holland,* A. Daniel,* and R. A. Maxwell. Research Dept., CIBA Pharmaceutical Products Inc., Summit, N. J.

Pressor responses to a series of phenylalkylamines are altered by guanethidine such that catechol derivatives are potentiated or not altered, amines possessing only β-hydroxylation are reversibly blocked and amines possessing no hydroxylation or hydroxylation in the para position only, are irreversibly antagonized. It has been found that guanethidine antagonizes the positive inotropic action of amphetamine and also antagonizes the vasoconstrictor action of amphetamine in the hind limb. Augmentation of the positive inotropic and vasoconstrictor actions of norepinephrine were less predictably demonstrated.

16

Pharmacology

FATE OF H^3-NOREPINEPHRINE IN ANIMALS. L.G. Whitby*, H. Weil-Malherbe* and Julius Axelrod. Natl. Insts. of Health, Bethesda, Md.

Using DL-8-H^3-norepinephrine (N) and methods specific for N and normetanephrine (NM) the metabolism and disposition of N was studied. Mice received 1 µgm. N i.v. and were killed at various times; 50% N disappeared in the whole mouse in 10 min., but thereafter the level fell slowly. The distribution of N and formation of NM in cat tissues were investigated after injecting 25 µgm. N/kgm. i.v. quickly or as an infusion. N distributed unevenly, heart,spleen and glandular tissues yielding the greatest amounts; tissues retained large quantities of N 2hr. after injection. NM was present in all tissues examined; considerable O-methylation had occurred within 2 min. Serial estimations in cat plasma showed a biphasic fall of N similar to the results in whole mice. It is concluded that the rapid fall of plasma levels is due to diffusion of N into tissues and O-methylation, and the subsequent slow fall to release from binding sites and metabolism. Regional distribution of N in brain after 2hr. showed high levels in the pituitary, and significant amounts in the hypothalamus, but negligible amounts in other areas.

17

Pharmacology

FORMATION OF ADENOSINE-3',5'-PHOSPHATE (3,5-AMP) BY PARTICULATE PREPARATIONS OF VENTRICULAR MUSCLE. F. Murad*, T. W. Rall*, and E. W. Sutherland. Western Reserve Univ. School of Med.,Cleveland, O.

Using washed particulate fractions from homogenates of ventricle and liver, the relative potency of several catecholamines in stimulating formation of 3,5-AMP was estimated. Compared with l-epinephrine the potencies using dog ventricular preparations were l-isopropylarterenol (l-iso) 9-12, l-epinephrine (l-epi) 1.0, l-norepinephrine (l-nor) 0.5-2, d-epinephrine 0.05; using dog liver preparations,the potencies were l-iso 4, l-epi 1.0, l-nor 0.15. In the presence of $1X10^{-6}$ M l-epi, the formation of 3,5-AMP by preparations of ventricle was increased nearly 2-fold (about half-maximal stimulation) and the addition of $1X10^{-5}$ M DCI reduced this increment by 80 to 95%. Using particulate preparations of ventricles from dog, rabbit and guinea pig, the formation of 3,5-AMP in the absence or presence of varying amounts of added l-epi was reduced by 10 to 30% when acetylcholine or carbachol was added. The possible relationship of this observation to those of Vincent and Ellis (The Pharmacologist 1, 62,1959) will be discussed.

18

Pharmacology

THE CONVERSION OF HYDROXYTYRAMINE TO NORADRENALINE BY BANANA HOMOGENATES. W. James Smith and Norman Kirshner (intr. by W.E. DeTurk). Dept. of Biochemistry, Duke Univ., Durham, N. C.

Previous work has shown that hydroxytyramine is a precursor of noradrenaline in the adrenal medulla (Kirshner, Pharm. Rev. 11, 350, 1959). Because of the lability of the animal enzyme system, and because of a report that noradrenaline is present in bananas (Waalkes, Science 127, 648, 1958), the latter were investigated as a possible source of the enzyme (s). It was found that hydroxytyramine was converted to noradrenaline by homogenates of green banana tissues. The peel, the pulp, the banana stalk rind, and the stalk pith were separately homogenized in 0.1% Cutscum solution in a Waring blendor and incubated with ATP, ascorbic acid, hydroxytyramine-C_{14}, and Krebs phosphate buffer in an atmosphere of 95% O_2, 5% CO_2 for one hour at 37°. The reaction was stopped by the addition of TCA; unlabeled hydroxytyramine and noradrenaline carrier were added, and isolated by ion-exchange chromatography. The noradrenaline was further identified by paper chromatography using two different solvents, and by recrystallization to constant specific activity. Enzyme activities of the various tissues were approximately the same and comparable to fresh adrenal homogenates. In contrast to the animal system, initial studies of dialysed extracts indicate that the reaction is not dependent on ATP or ascorbic acid.

19 **Pharmacology**

THE EFFECTS OF RESERPINE, NOR-ADRENALINE AND INHIBITORS ON THE METABOLIC RATE OF RATS. G. E. JOHNSON AND E. A. SELLERS (INTR. BY G. H. W. LUCAS.) DEPT. OF PHARMACOLOGY, UNIV. OF TORONTO, TORONTO, CANADA.

FOR A VARIABLE BUT SHORT PERIOD AFTER THE ADMINISTRATION OF RESERPINE OR OF NOR-ADRENALINE TO ALBINO RATS AN INCREASE IN METABOLIC RATE (OXYGEN CONSUMPTION) IS OBSERVED. THIS STUDY DESCRIBES THE EFFECTS OF PRETREATMENT WITH SPECIFIC INHIBITORY SUBSTANCES ON THIS PHENOMENON. THE INHIBITORY SUBSTANCES EMPLOYED WERE DIBENZYLINE (ADRENERGIC ALPHA RECEPTORS), IPRONIAZID (MONOAMINE OXIDASE), AND PYROGALLOL (O-METHYL TRANSFERASE). PRETREATMENT WITH DIBENZYLINE ABOLISHED THE INITIAL RISE IN METABOLIC RATE NORMALLY PRODUCED BY RESERPINE, SUGGESTING THAT THIS RISE IS DUE TO THE RELEASE OF ADRENALINE AND/OR NOR-ADRENALINE. PRETREATMENT WITH IPRONIAZID PROLONGS THE INCREASE IN METABOLIC RATE ELICITED BY NOR-ADRENALINE WHILE PRETREATMENT WITH PYROGALLOL HAS LITTLE EFFECT. THESE RESULTS TAKEN ALONE SUGGEST THAT O-METHYL TRANSFERASE PLAYS LITTLE OR NO PART IN THE DETOXIFICATION OF NOR-ADRENALINE WHILE MONOAMINE OXIDASE PLAYS A PRIMARY ROLE. (PARTIALLY SUPPORTED BY THE DEFENSE RESEARCH BOARD OF CANADA).

20 **Pharmacology**

BILIARY EXCRETION OF DL-EPINEPHRINE METABOLITES. Elwood H. LaBrosse and George Hertting. (Intr. by S. S. Kety). National Institutes of Health, Bethesda, Md.

DL-Epinephrine-7-H^3-d-bitartrate was administered intravenously to four male Sprague-Dawley rats. Of the total radioactivity excreted into the bile and urine, 13.5 ± 6.4% was found in the bile. The radioactivity in the bile was entirely localized in two constituents. By paper chromatography before and after hydrolysis, these constituents have been identified as metanephrine glucuronide (ME-glucuronide) and 3-methoxy-4-hydroxyphenylglycol sulfate (MHPG-sulfate) (Axelrod, Kopin and Mann, Biochem. Biophys. Acta, in press). After the administration of bile containing H^3-MN-glucuronide and H^3-MHPG-sulfate into the duodenum of rats, only about 2.5% of the administered radioactivity was excreted in the urine in 4 hours, and ME-glucuronide was the sole radioactive constituent in this urine. These results indicate that a portion, about 13%, of epinephrine metabolites are excreted in the bile of rats; only a very small portion, about 2.5%, of the biliary metabolites are absorbed in the intestine and excreted in the urine within four hours after duodenal administration.

21 **Pharmacology**

METABOLISM OF NOREPINEPHRINE BY RAT BRAIN AND HEART. J.R. Crout, C.R. Creveling, and D. Caton (intr. by A. Sjoerdsma.) Natl. Heart Inst., Bethesda, Md.

Meta-O-methylation is a major pathway of metabolism for circulating norepinephrine (NE), but the importance of catechol-O-methyl transferase (COMT) relative to monoamine oxidase (MAO) in degrading endogenously formed NE in tissues is unknown. This problem was studied by measuring effects of single i.p. injections of MAO inhibitors and of pyrogallol, a COMT inhibitor, on the NE content of rat brain and heart. The MAO inhibitors usually produced significant increases in the tissue content of NE (peak at 10-18 hrs.) but pyrogallol produced no changes for periods up to 10 hrs. The *in vivo* accumulation of injected NE in rat heart relative to plasma was seyeral times greater in iproniazid treated animals than in controls but in pyrogallol-treated rats was less than in controls. The ratio of MAO activity to COMT activity of rat brain and heart, *in vitro*, was 25-50 times that for liver. These studies indicate that MAO inhibitors allow both endogenously formed and exogenously administered NE to accumulate in tissues, whereas a COMT inhibitor does not. The high COMT activity of liver is confirmed, but the quantitative significance of this enzyme relative to MAO in metabolizing NE in rat brain and heart is questioned.

22 **Pharmacology**

INHIBITION OF UPTAKE OF EPINEPHRINE IN BRAIN SLICES BY RESERPINE. Elwood Titus, Cedric W. M. Wilson* and Hans J. Dengler*. Natl. Heart Inst., Bethesda, Md.

Studies of the uptake of catecholamines by brain slices have been undertaken in the hope of clarifying the nature of the binding sites. Tissue slices from normal and reserpinized cats were incubated for 1 hr. in a 1:1 mixture of cat serum and Krebs-Ringer bicarbonate, pH 7.4, containing 15 μg/L of DL-epinephrine-7H^3. Epinephrine was determined by scintillation counting of acetic acid eluates from alumina columns. Three hrs. after i.p. injection of 3 mg reserpine/kg the ability of several tissues to concentrate epinephrine diminished. Ratios of epinephrine concentration in tissue/concentration in medium for normal and reserpinized animals respectively were: cortex 1.7, 0.35; thalamus, 1.5, 0.46; hypothalamus 1.7, 0.7; cerebellum 0.7; 0.4; liver 0.78, 0.17; skeletal muscle, 0.52, 0.15. The concentrating mechanism appeared to approach saturation at higher external concentrations.

193. NEUROPHYSIOLOGY—Electric Potential

1 **Physiology**

STABLE STATES AND OSCILLATIONS OF SKELETAL MUSCLE. G. Falk and J. F. Landa*. Univ. of Wash. Sch. of Med., Seattle, Wash.

Skeletal muscle in the resting state has a high ratio of chloride: potassium permeability. Frog sartorii fire repetitively after a single shock in a medium in which chloride has been replaced by anions which do not carry charge across the membrane. Spike activity is followed by a plateau phase in which the membrane potential is set at -35 to -45 mv. Spontaneous repolarization is aperiodic, resembling that in other cells in prolonged action potentials, or is oscillatory with negative damping. Both duration and growth of oscillations are sensitive to external potassium concentration. In the absence of external potassium fibers depolarize spontaneously, remain depolarized indefinitely at the potential of the plateau or oscillate in the region of the plateau. The oscillations have the characteristics of relaxation oscillations with a period usually of many seconds. When potassium is added to the medium, fibers repolarize promptly. The effects of potassium on duration of the prolonged action potentials are independent of changes in the resting potential. It is concluded that removal of the chloride shunt reveals another stable state of membrane potential. The effects of potassium could be explained on the basis of anomalous rectification as well as dependence of conductance for this ion on its external concentration. (Supported by Grant B-895, NINB)

22 **Physiology**

ELECTRICAL ACTIVITIES OF SINGLE RANVIER NODE OF FROG NERVE. H.Ooyama and E.B.Wright, Coll. of Florida, Gainesville, Fla.

Electrical activity at the single Ranvier node has been studied by both voltage clamp and potential observations. Sudden depolarization by voltage clamp produces abrupt initial increase of inward current, due to sodium permeability increase. The rising phase is slower than the falling phase and the duration is far less than an action potential. With increasing depolarization this current becomes first oscillatory then the oscillations fuse together appearing similar to the currents obtained from the squid giant axon. These sodium current patterns are unaffected by high concentrations of potassium. In excess potassium a greatly prolonged action potential is elicitable due to increased inward potassium current. This potassium current under voltage clamp increases very slowly at first then abruptly to a higher level during a weak depolarization. It decays slowly during depolarization, and the recovery to the normal level after depolarization is withdrawn is also far slower than the sudden rise. The lack of effect of potassium on the initial sodium current though surprising in view of the drastic changes in the potential records, is predicted by and supports the existence of two independent processes underlying excitation, sodium carrier and potassium carrier activities mediated by the membrane potential difference. (Supported by NSF Grant 6991.)

XUM

3 *Physiology*

SEGMENTAL AND CROSSED EPHAPTIC TRANSMISSION IN CRAYFISH LATERAL GIANT AXONS. A. Watanabe*, T.G. Smith* and H. Grundfest, P & S, Columbia U., New York.

Unpolarized transmission across segmental septa is electrical, by local circuit current from pre-spike. Post-ephaptic potential (p.e.p.) initiates post-spike after 100-500 μsec delay. P.e.p.'s are produced electrotonically, without latency, in either direction. Their amplitude, proportional to the pre-spike, is independent of post-segment membrane potential. They add slightly to post-spike height. Septal membrane is inexcitable by p.d. of 70 mv. Electrotonic spread, unrectified, is attenuated at septa by 1/4-1/2, indicating resistance matching of septa, pre-, and post-segments. Unpolarized electrotonic spread, but much more attenuated, also occurs between lateral axons. Cross-excitation between axons, by spikes, may occur in either direction (delay about 700 μsec) causing reciprocally repetitive discharges. Transmissional failure at either axon reveals prepotentials far below critical firing amplitude. The sites of this crossed ephaptic transmission are probably at segmental commissural branches between lateral axons.

4 *Physiology*

OSCILLATORY HYPERPOLARIZING RESPONSES IN LOBSTER MUSCLE FIBERS. J. P. Reuben, R. Werman and H. Grundfest (intr. by M.J. Frumin). P&S, Columbia U.N.Y.

Lobster muscle fibers, hyperpolarized 60 ± 10 mv in physiological saline, frequently showed discontinuous rise in membrane potential ("hyperpolarizing response"). Conductance measurements using pulses, p.s.p.'s, or time constant reveal a concomitant increase (3 or 4-fold) in resistance, due to decreased K^+ conductance, without apparent change in membrane electrode properties. Excess hyperpolarization by strong anodal currents, or sometimes by the hyperpolarizing response itself may increase conductance above resting value. Prolonged anodal currents therefore may cause oscillations of potential due to alternating high and low membrane resistance states. Anodal or cathodal pulses can terminate hyperpolarizing responses, but decrease resistance by different mechanisms. Ionic changes that alter K^+ conductance (added alkali earth cations; increased or decreased K^+), and increased Cl^- conductance during inhibitory synaptic activity block the hyperpolarizing response.

5 *Physiology*

TEA AS A SUBSTITUTE FOR Ca^{++} IN LOBSTER MUSCLE FIBERS. R. Werman and H. Grundfest (intr. by F. A. Mettler). P & S, Columbia U., New York.

Resting conductance of lobster muscle fibers is greatly decreased in the presence of alkali earth cations. The membrane becomes hyperpolarized and relatively insensitive to change of outside K^+. These effects, ascribable to decreased K^+ permeability, also account for the change of gradedly responsive membrane to one producing all-or-none, overshooting, prolonged, decrementlessly propagating spikes. Removal of Ca^{++} markedly increases resting conductance, depolarizes the fiber, and secondarily leads to reduction of response height. Tetraethylammonium ions (TEA) prevent the changes produced by removal of Ca^{++}. In the presence of Ca^{++}, TEA increases membrane resistance, causes hyperpolarization, produces all-or-none spikes and protects against depolarization by excess K^+. Quantitatively, TEA is about 20 times more effective than is Ca^{++}, but is less so than are Ba^{++} or Sr^{++}. Since the mechanisms of their action appear to be identical, it is of interest that the Stokes-Einstein ionic radii of TEA and alkali-earth cations are similar.

6 *Physiology*

EFFECT OF TEMPERATURE ON SPONTANEOUS AND INDUCED ACTIVITY OF PERIPLANETA AMERICANA VENTRAL NERVE CORD Paraskevi J. Mavridis and R. C. Sanborn (intr. by W. A. Hiestand) Purdue University, Lafayette, Ind.

Isolated female cockroach cords were subjected to temperatures ranging from 10 to 32° C. At temperatures (as measured with a thermistor in contact with the cord) below 25° individual fibers show both increased frequency and magnitude of spontaneous activity. At temperatures below 12°, the activity decreases markedly and at 10° only isolated fibers fire at approximately 1 sec. intervals. As the temperature is increased above 25°, activity remains constant until 30-31°, above which temperature there is again decreased spontaneous activity. Within the above limits the changes are reversible, but show an apparent "hysteresis" in that a chilled cord displays higher activity at each temperature as it is rewarmed than it did during cooling. The threshold and synaptic delay of cords cooled below 25° increase in an apparently smooth fashion. Below 14° cords are not excitable by stimuli of at least 10 times the threshold voltage at 25°.
(Supported by grants from the National Science Foundation and from the Purdue Research Foundation).

7 *Pharmacology*

PRE- AND POSTSYNAPTIC SLOW POTENTIALS IN VIRUS-INFECTED SYMPATHETIC GANGLIA OF THE RAT. Jacob Zabara* and John Dempsher. Dept. of Pharmacology, Univ. of Penna. Med. School, Phila., Pa.

It was previously reported that two kinds of spontaneous potential changes were observed in excised rat superior cervical ganglia following infection with pseudorabies virus, a slowly changing potential, the slow potential, on which was superimposed a rapidly changing potential, the spike. It was suggested that the presynaptic slow potential originated in the presynaptic nerve endings, served as a focus for depolarization of the preganglionic axon and represented the electrical event accompanying interaction between excitatory and inhibitory systems at the presynaptic nerve endings. Present observations suggest that transmission of excitation occurs when the slow potential in the presynaptic nerve endings induces a similiar potential change in the postsynaptic neuron; in addition, these observations suggest that the postsynaptic event is turned on and off in the presynaptic nerve endings. For example, the following chemical agents shown to exert effects upon the presynaptic nerve endings affected both potentials simultaneously and synchronously. Physostigmine and acetylcholine initially increased then decreased the pre- and postsynaptic slow potentials simultaneously and synchronously; tubocurarine, gamma aminobutyric acid and epinephrine decreased both potentials simultaneously and synchronously.

194. NEUROPHYSIOLOGY– Peripheral Nerves

1 *Physiology*

MOTOR NERVE FIBRE CONDUCTION IN TICK PARALYSIS M.F. Murnaghan (introd. by L.P. Dugal) Dept. Pharmacology, University of Ottawa, Ottawa, Ontario, Canada.

In a previous investigation in this laboratory it had been concluded that the paralysis produced in dogs by the wood tick, Dermacentor andersoni Stiles, is probably due to a conduction block in the terminal motor nerve fibres. In the present study conduction in motor nerve fibres was investigated in normal and paralysed dogs by stimulating the 6th. lumbar ventral root and recording the action potentials from the peroneal nerve. While the amplitude of the potential recorded from α fibres was reduced about 3 times by moderate paralysis, that from β and γ fibres was reduced approximately 30 times and that from δ fibres seemingly even more so. Furthermore moderate paralysis significantly reduced the conduction velocity particularly in β, γ and δ fibres. In severe paralysis only a single potential of small amplitude, probably originating from α fibres, was recorded. It is concluded that tick paralysis is due to a conduction block in motor nerve fibres and that the 'tick toxin' exhibits a predilection for the smaller diameter motor nerve fibres. (Supported by a grant from the Dept. Agriculture, Ottawa, Canada.)

195. NEUROPHYSIOLOGY–Reflexes

2 *Physiology*

ANALYSIS OF GLUCOSE-LACK IN SYMPATHETIC GANGLIA. M.G. Larrabee, F.J. Brinley Jr.*, P.Horowicz*, J.Klingman* and G.Looney*. Biophysics Dept. Johns Hopkins Univ.

As previously reported, superior cervical ganglia excised from rats respond to preganglionic stimulation for many hours at 37°C in solutions containing glucose, but lose capacity to respond after 2 hr without glucose, with only slight slowing of oxygen uptake. In recent experiments ATP content (luciferase method) was no lower in ganglia kept 3 or 13 hr without glucose than in ganglia freshly excised or kept 3 hr with glucose. K content (measured with K^{42}) declined only about 10% after 2 hr without glucose and K efflux was accelerated about 15%. Thus no evidence was obtained that glucose-lack interferes significantly with energy production or K concentration. Perhaps glucose is a source for continual replacement of an essential compound. In agreement is the finding (using C-14) that only 25% of the carbon from glucose is found as CO_2, 25% as lactate, and 10% as tissue constituents, leaving 40% attributable to undetected materials diffusing into the bathing fluid and lost during chromatography of this fluid. (Aided by NIH Grant B-702.)

3 *Physiology*

THE IMPULSE AT THE END OF A NERVE. Dexter M. Easton, Division of Physiology, Florida State University, Tallahassee, Florida.

A hydraulic holding technique suitable for stimulating a nerve and for recording impulses was devised. Each cut end of a frog nerve was pulled into a separate glass tube. The nerve was at all times surrounded by Ringer's solution. A chlorided silver wire in the tube served as the stimulating electrode or as the active recording lead. The indifferent lead was in the surrounding fluid. Spikes were monophasic positive 1 to 5 mm from a freshly cut end, while a trailing negativity developed at greater distances and increased with time after the cut. The diphasicity of the spike implies invasion of the impulses into the terminal during "healing" of the nerve. During a train of stimuli, the freshly cut end showed a positive-going maintained potential (DC recording) and a negative-going slow potential following the train. The sequence of changes was approximately reversed at the healed end. Slow positivity at the cut end and negativity at the healed end during a train both imply a decrease of injury current to the cut end. The hydraulic holding technique is recommended as superior to conventional methods of recording from whole nerve. Injury to the preparation occurs less readily and the potentials generated by the impulse currents are in some respects more consistent with the signs of potentials recorded via intracellular microelectrodes. (Grant USPH B927)

4 *Pharmacology*

THE ACTION OF ACETYLCHOLINE ON CONDUCTION IN MAMMALIAN NON-MYELINATED FIBRES. Christine J. Armett* and J.M. Ritchie. Dept. of Pharmacology, Albert Einstein College of Medicine, New York.

It is generally agreed that nerve fibres are insensitive to acetylcholine. Recently, however, it has been suggested that acetylcholine may have some effect on the after-potentials of the spike in mammalian non-myelinated fibres (Douglas, W.W. & Ritchie, J.M., J. Physiol., in press). In the present experiments the sucrose gap technique has been used to study the action of acetylcholine on the C potential of the desheathed rabbit vagus nerve. In concentrations of 10^{-4} w/v, or less, acetylcholine has no effect on the spike or after-potential. However, in a higher concentration ($3x10^{-4}$ w/v) acetylcholine causes a reduction of 20-40% of the spike amplitude and a depolarization of the fibre. These effects are accompanied by characteristic changes in the after-potentials, the positive after-potential becoming much more pronounced; these changes seem to be a result of the depolarization because they are reversed by applying hyperpolarizing currents to the nerve and because they can be mimicked by applying depolarizing currents to the nerve in Locke's solution. A concentration of 10^{-3} w/v causes a greater depolarization and may block conduction. These effects appear very soon after acetylcholine is applied (less than 1 minute) and are rapidly reversed when the preparation is returned to Locke's solution.

1 *Physiology*

Reciprocal Innervation of the Paravertebral Musculature. John N. Eble (intr. by I. M. Korr) Kirksville College of Osteopathy and Surgery, Kirksville, Mo.

The simultaneous reflex response patterns of the paravertebral muscles in the lumbar region in acute spinal rabbits (T1-T2) to a variety of visceral and somatic stimuli were recorded electromyographically. The muscle groups studied were Multifidus, Sacrospinalis, Intertransversarii, Psoas major and in addition the Rectus abdominis. There was little or no interrelation between the Multifidus on opposite sides whereas a stimulus which reflexly excited the Intertransversarii of one side inhibited the activity of the Intertransversarii of the other side. Thus, the Intertransversarii muscles of opposite sides function as antagonists while the Multifidus do not. Further, the Multifidus of one side and the Intertransversarii of the other side function as antagonistic pairs but the Multifidus and Intertransversarii of the same side are relatively independent of each other. Thus, pinching the skin of the dorsum to the right of the midline reflexly excites the underlying Multifidus and inhibits the Intertransversarii of the left side. This reciprocal relationship is such as to facilitate lateral rather than ventral flexion. The threshold for inhibition of pre-existing activity of antagonistic muscle groups is lower than that for the initiation of activity of the agonists.

2 *Physiology*

EFFECTS OF LATERAL RECTUS MUSCLE CONTRACTION UPON INTRAOCULAR PRESSURE. N. Krishna (intr. by S. Y. Botelho), Dept. Physiol., Grad. Sch. of Med., Univ. of Pa., Phila. Contraction of the right lateral rectus muscle was produced by means of direct and indirect electrical stimulation in cats which were anesthetized with nembutal. Intraocular pressures of both eyes, femoral arterial pressure and respiratory rate were recorded by means of appropriate strain gauge transducers, which were connected to an ink writer. Observations were made of changes in pupil size, activity of ciliary muscle, movements of nictitating membrane and movements of both eyes. In order to correlate changes in intraocular pressures with the degree of activity of the lateral rectus muscle, the stimulus parameters were varied and the resultant tension developed by the muscle was recorded. Data were obtained both before and after separation of the ipsi- and contralateral extraocular muscles from their origins and insertions. Results of these experiments indicate that unilateral contraction of the lateral rectus muscle alone not only produced an ipsilateral increase in intraocular pressure but also a slight initial rise followed by a more marked fall in intraocular pressure of the contralateral eye. Possible mechanisms to explain these findings will be discussed.

3 *Physiology*

FORCE PATTERNS NECESSARY FOR REFLEX CONTROL OF MOVEMENT. Lloyd D. Partridge (intr. by J.F. Fulton). Yale Univ. School of Med., New Haven, Conn.

Measurements from motion picture frames recording an attempted abrupt, small-angle, horizontal movement of the forearm, with elbow fixed, provide information on the velocities and accelerations attained in this relatively simple voluntary movement. If the arm is considered to move as a rigid body, force calculations can be made by use of moment-of-inertia estimates. A force graph so constructed shows that the sum of forces rotating the limb take on a complex oscillatory pattern in an attempted step movement. The primary accelerating forces reach their peak and end early in the movement, being followed by opposing forces retarding the movement. Analysis of the force patterns indicate that they would be compatible with the supposition that, even in rapid movements, a gamma regulated, adapting muscle-spindle response, through the monosynaptic reflex, can control the limb position. (Aided by USPHS Grant B-2355).

XUM

4 *Physiology*

RELATION BETWEEN REFLEX AND REACTION TIME. Peter V. Karpovich Springfield College, Mass. and Michio Ikai*, Univ. of Tokyo.

It is generally assumed, in athletics, that a person with faster reflexes has a faster reaction time. The purpose of this study was to test this assumption. Twenty-nine subjects, from 17 to 40 years of age, were used. They led sedentary lives, being inmates of a county jail. The time of the knee jerk reflex and the reaction time of the kick of the foot were compared. In both tests the subject was sitting, and the tested leg was secured in a swing which had an electrogoniometer attached concentrically with the hinge of the swing, so that the knee action, in degrees, could be recorded on an oscillograph. (Fed. Proceedings, 18:311, 1959) No statistically significant correlation was found between the reflex and reaction times, but there was a significant correlation ($r = .55$) between the speed of the reflex and reaction movements. (Speed was expressed in degrees per sec.) A high correlation ($r = .95$) was found between the amplitude and the speed of the reflex movement, but the same measures for the reaction time gave an $r = -.16$ (not significant). It must be concluded that, at least in sedentary persons, the speed of a reaction cannot be judged by that of a reflex. (Supported by grants from the U. S. Army Medical Research and Development Command, Office of the Surgeon General; and the American College of Sports Medicine.)

5 *Physiology*

HISTOCHEMICAL INDICATION OF RECIPROCAL INHIBITION. Ernest Retzlaff*, Benjamin Pasamanick*, Joan Fontaine*, Chauncey Leake. Research Division, Columbus Psychiatric Institute, Ohio State University, Columbus, Ohio.

Neurohistological and neurophysiological studies of the giant Mauthner's cells which lie in the floor of the medulla oblongata of teleosts demonstrate that these two motor neurons are interrelated both structurally and functionally. This relationship is accomplished by a system of VIIIth nerve afferents and by interconnecting axo-axonal collaterals.

It has been possible by unilateral stimulation of the VIIIth nerve roots to alter the intracellular chemical state of the Mauthner's cells as indicated by a differential staining reaction to activated protargol. The cell homolateral to the applied VIIIth nerve root stimulus displays deeply colored dendrites and cell body while the axon hillock region appears nearly colorless. In contrast, the contralateral Mauthner's cell is stained in a reverse manner so that the dendrites and cell body are nearly colorless and the axon hillock region is deeply stained.

This histochemical reaction of the protargol stained Mauthner's cells to the VIIIth nerve root stimulation lends support to an earlier proposal (J. Comp. Neur. 1954, 101:407-446) that such staining indicates the activity state of these neurons; namely, reciprocal excitation and inhibition. (Supported in part by N. I. H. Grant MY 3271.)

6 *Physiology*

OCULAR COMPRESSION CAUSING C.N.S. REACTIONS OTHER THAN THE OCULO-CARDIAC REFLEX. E. Aserinsky and D. DeBias (Intr. by D. J. Sandweiss.) Dept. Physiol., Jefferson Med. Coll., Phila., Pa.

Pressure on the eyeballs induces the "oculo-cardiac reflex," and is also known to affect respiratory and gastrointestinal movements. The procedure has widespread use as the basis for determining vagal tone because the vagal center is believed to be the principal site of stimulation. Observations on 11 dogs suggest that the "oculo-cardiac reflex" is simply one manifestation of a generalized C.N.S. response which also involves the somatic nervous system. Manual pressure exerted on both eyes for 30 seconds elevated intracranial pressure by 12mm-16mm H_2O, and depressed the heart rate in surgically anesthetized animals by an average of 45 beats/minute. With lighter anesthesia, the rate was reduced by only 24 beats/minute. During the ocular compression, a crossed extensor reflex induced by stimulation of the sciatic nerve was either partially or completely inhibited. Division of either one of the vagi had no marked effect on the responses to pressure on the eyes whereas double vagotomy either inverted the usual cardiac response or reduced it, but had no effect on the suppression of the crossed extensor reflex.

196. NEUROPHYSIOLOGY– Sensory Systems

1 *Physiology*

STUDY OF NEURONAL ACTIVITY IN THALAMIC NUCLEI, WITH MULTIPLE MICROELECTRODES. K. Negishi (Introd. by M. Verzeano.) Dept. of Biophysics, UCLA.

The activity of neurons in the n. reticularis and lat. geniculate was studied by means of multiple microelectrodes, in cats in the waking state and under barbiturate anesthesia. Recordings were obtained, simultaneously, in some experiments with three, in other experiments with four microelectrodes. The tips of the microelectrodes (1 to 5 μ in diameter) were displayed along a straight line and separated by distances of 50 to 300 μ. The tip of each microelectrode was recording, extracellularly, the activity of one single neuron. Stimulation with brief flashes of light produced, in both nuclei, patterns of response which exhibited definite spatial and temporal relations between the activity of various neurons. These patterns could be modified by changing the frequency of stimulation. In the n. reticularis the responses to light were localized in the caudal part and their average latencies were longer than in the lat. geniculate. These findings indicate that sensory information arrives in "diffuse" as well as in specific systems and that it may be represented by patterns of spatial and temporal relations between the activities of the neurons in these networks. (Supported by a grant from USPHS, Grant B-649).

2 *Physiology*

THE EFFECT OF SENSORIMOTOR CORTEX AND MESENCEPHALIC RETICULAR FORMATION ON UNIT ACTIVITY AND EVOKED POTENTIAL IN THE DORSAL COLUMN NUCLEI. Robert Siminoff. (Intr. by C. D. Clemente.) School of Med., Univ. of California, Los Angeles.

A flexible microelectrode was used to record 103 units in the dorsal column nuclei of Flaxedilized cats. Natural peripheral stimulation as well as electrical stimulation of the dorsal column was used. The latencies of unit firing varied from 1.5 to 30 msecs. when the dorsal column was directly stimulated. The relationship between unit activity and the evoked potential was studied. Stimulation of the sensorimotor cortex and mesencephalic reticular formation did not produce any change in the evoked potential or the spontaneous and naturally evoked activity of the units. Stimulation of the sensorimotor cortex did not produce any change in the electrically driven unit activity. However, stimulation of the mesencephalic reticular formation inhibited 5 of the units when driven at just threshold voltage.

3 *Physiology*

THORACIC STRETCH-RECEPTORS IN THE CRAYFISH. R. L. C. Pilgrim* and C. A. G. Wiersma. Calif. Inst. Tech., Pasadena, Calif.

In investigations on the presence of muscle sense organs in Crustacea, Alexandrowicz noted that anteriorly in the thorax a small number of N-cells appears to be the morphological counterpart of the posterior thoracic and the abdominal stretch receptors. The physiological function of the N-cells has not yet been reported. By leading from the nerve fibers of these cells in the crayfish, signals were recorded when stretch was applied to the muscles with which they are associated. So far, six separate cells have been made to respond. The locations of five of them correspond fairly well with those given by Alexandrowicz for the lobster. Most cells produce signals strongly reminiscent of those of the slowly-adapting abdominal muscle receptors but at least one resembles more closely the fast. The slow N-axons appear to enter the cord by separate roots, but the fast N-axon enters with one of the slow. The responses have also been obtained in isolated preparations. There appear to be considerable differences between the functions of various N-cells in the intact animal. (Supported by grants from Nat.Acad.Sci., and N.S.F.)

4 *Physiology*

A MICROELECTRODE STUDY OF CEREBELLAR FORELIMB AFFERENTS. Robert G. Grossman, George Moushegian, Lawrence J. Viernstein and Allen Rupert (intr. by Robert Galambos.) Walter Reed Army Institute of Research, Washington, D.C. and Johns Hopkins/Appl. Physics Lab., Silver Spring, Md.

A microelectrode study of single units representing the ipsilateral forelimb and neck in the restiform body and cerebellum was carried out in 36 cats anesthetized with pentobarbital. These units comprised 75% of the 118 somatic afferents studied. The shoulder was represented by the largest number, followed by the digits, wrist, elbow and neck. Most units were sensitive to deep stimulation, fewer to both deep and cutaneous, and a few to cutaneous only. Increased discharge rate regularly followed movement of one or two joints in a single direction. A few units were affected by pressure applied to the forelimb, simulating weight-bearing. The firing rate of these units was increased by manipulation of the shoulder and decreased by pressure on the paw pad. Forelimb units had mean discharge frequencies below 100/sec. and adapted slowly over several minutes. Interspike intervals varied from 5 to 200 msec. Proprioceptive stimulation produced a large shift in the most commonly occurring interspike interval and a small shift in the range of intervals.

5 *Physiology*

THE RELATION OF NASAL MEMBRANE FUNCTION TO OLFACTORY ACUITY. Robert A. Schneider (intr. by A.N. Taylor) Dept. Med., Univ. of Okla. Hosps. and Okla. Med. Res. Found., Okla. City.

863 determinations of perception threshold for Citral and accompanying degrees of nasal obstruction, swelling, color and wetness were made on 8 subjects. Thresholds were determined in a temperature-humidity controlled olfactorium where odorant concentrations could be widely varied. Swelling, secretions and obstruction were estimated on a 0 to 4 scale and color on a color scale. Olfactory acuity was impaired in 2 situations. 1- in the presence of high degrees of nasal obstruction so that relatively little of the odorant containing air could be brought into the nose. 2- in the absence of obstruction when the mucosa was pale, dry and shrunken. During intermediate degrees of nasal obstruction when substantial amounts of air could be drawn into the nose relatively high degrees of swelling, redness and wetness favored olfactory acuity. Thus smelling was best performed in the presence of a red, swollen, wet mucosa as long as swelling was not of such a degree as to substantially block air passage. The evidence suggests that contact of odorant with nerve endings is facilitated by the greater warmth and humidifying effect of nasal engorgement during hyperfunction of the "air conditioning" apparatus of the nose. (Supported by grant B-286C6, N.I.H., sec. on Neur. Dis. and Blindness).

6 *Physiology*

EARLY AND LATE RESPONSES AT CORTEX TO LOCAL ELECTRICAL STIMULATION OF CAT RETINA. Frances R. Grimm* and R. W. Doty Dept. of Physiology, Univ. of Michigan, Ann Arbor, Michigan.

Photically evoked cortical potentials are little changed hours after removal of vitreous humor. Electrical pulses of 1 msec. applied to exposed dorsal retina through electrodes 1-2 mm apart evoke two types of cortical response. The "early" responses appear in 3-5 msec. at restricted cortical loci dependent upon location of retinal stimulus. They are morecommon and larger in central than medial portions of dorsal marginal gyrus. Wave-form is identical with that to optic nerve stimulation. Responses of 2 msec. latency, still localized cortically, can be obtained with electrodes close to optic disc. Beyond the cortical zone of early response a slow, "late" wave appears after 20-110 msec., often highly similar to photically elicited responses. Optic tract potentials indicate much of the late response is elaborated intraretinally. Late responses may be the same at a cortical point for stimuli in nasal and temporal retina. Early and late responses are reciprocal in cortical location, but may fail to interact when converging on a cortical point from different retinal loci. Photically and electrically elicited responses are mutually depressive. Trigeminal neurotomy does not affect results, nor does use of animals immobilized with pontine level sections in place of the usual Nembutal anesthesia. (USPHS Training Grant 2B-5252)

7 *Physiology*

HIGHER AUDITORY CENTERS AND THE DL FOR SOUND INTENSITIES. R. E. Oesterreich* and W. D. Neff. Lab. of Physiological Psychology, Univ. of Chicago, Chicago, Ill.

Cats were trained by the shock avoidance technique to respond to a change in intensity of a tone. Difference limens (DLs) were determined before and after bilateral cortical ablations ranging in size from auditory areas I, II, and Ep to I, II, Ep, somatic area II, anterior suprasylvian gyrus, and insular-temporal region. In some cases, cortical ablations were combined with bilateral subcortical extirpation of the inferior colliculus (IC). In other cases the brachium of the inferior colliculus (BIC) or the lateral lemniscus, just caudal to the inferior colliculus, was sectioned bilaterally. DLs were unchanged after ablations of either cortex or IC. A combined cortical and IC ablation resulted in a small change in the DL for intensity. Threshold shifts after section of the BIC varied from 0 to 10 db depending on the extent of the lesion. The results of this study suggest that intensity discriminations may be mediated by auditory centers at tectal or medullar levels. (This research was supported by grants from the Office of Naval Research and the Air Force Office of Scientific Research.)

8 *Physiology*

EFFECT OF PERIPHERAL NERVE STIMULATION AND MUSCLE ACTIVATION ON RESPIRATION AND BLOOD PRESSURE NOCICEPTIVE REFLEX PATTERN. J.H.Perryman, S.Katz* and L.J.Goldberg*. N.Y.Univ. Coll. Dent.

The measurement of a nociceptive reflex response in the cat based on alterations in respiratory pattern and blood pressure is complicated by the effect of muscle afferent activity not necessarily related to pain. The greatest alterations in respiration and blood pressure in the lightly anesthetized or decerebrate preparation occur when stimulation to the skin is strong enough to produce a muscle response or when peripheral nerve stimulation produces some limb muscle contraction. Stimulation of the peripheral end of lumbar ventral roots at moderate intensity can produce changes in respiratory pattern and blood pressure not related to circulatory feedback, joint movement, stretch or ischemic pain. To determine how much of the nociceptive reflex response is due only to afferents unrelated to muscle, the effect of direct stimulation of the sciatic before and after muscle denervation, stimulation of the saphenous, mandibular nerve and tooth pulp were compared. The level of anesthesia is an important factor in the magnitude of response, and noxious or direct peripheral nerve stimulation may produce only moderate to slight effects when not reinforced by muscle feedback.
(Supported by research grant B-1347 from the Natl. Inst. of Neurological Diseases and Blindness, P.H.S.)

9 *Physiology*

PHYSIOLOGIC RESPONSES OF THE HUMAN SENSORY THALAMUS. Frank R. Ervin and Vernon H. Mark (intr. by E.L. Gasteiger), Mass. Gen. Hosp. and Harv. Med. Sch., Boston, Mass.

Electrodes stereotactically placed to relieve the pain of terminal CA have permitted the investigation of thalamic function in 15 conscious patients. Electrical stim. of peripheral nn., produced less striking potentials than physiologic stimuli. Thalamic stim. at fixed parameters produced discretely localized, contralateral, nonspecific subjective responses of "tingling". Distribution of threshold subjective responses was roughly somatotopic; the head and face represented 5-8 mm lat. to midline, the upper extremities 8-12 mm. and the lower extremities 12-15. Increased power input produced spread of the subjective sensation by anatomic contiguity (e.g. hand to arm). Variation of frequency above 20/sec could shift the locus of sensation. Frequencies below 20/sec evoked contralateral motor movements which followed the stimulus frequency. Scalp recording during thalamic stimulation demonstrated short latency potentials maximal over the ipsilateral central fissure. Repetitive stim. @6-9/sec produced in some positions recruiting or augmenting responses also maximal ipsilaterally. Both single evoked potentials and repetitive responses could be evoked at parameters below the subjective threshold of the subject without production of obvious changes in behavior. (Supported by grants from NINDB and UCP).

XUM

10 **Physiology**

Spatial Determinants of Visual Excitability. I.H. Wagman and W.S. Battersby. Mount Sinai Hospital, New York City.

Neuro-retinal rather than central processes are usually invoked to explain psychophysical changes in visual sensitivity produced by altering target size. In the present study, thresholds were measured at various intervals between "test" and "conditioning" flashes presented either to the same(monocular) or to opposite (binocular) eyes. The 40' test target (5msec.) was centered within a conditioning target (500 msec.) whose size was either 40', 1° 20', 2°, or 4° 40' of visual angle. With the largest conditioning target, increments in threshold over time were small with binocular, much greater with monocular stimulation. As the conditioning target was decreased in size, increments in threshold increased markedly. The magnitude of this change was much greater for binocular than for monocular stimulation. "Peripheral" limitations of visual excitability were estimated for each size conditioning target by subtracting binocular from monocular thresholds. The resultant excitability changes could then be described by a single function for all conditioning target sizes. This finding suggests that visual excitability is limited more by central than by peripheral processes when test and conditioning flashes approach each other in size. (Supported, in part, by USPHS Grants Nos. B-174 and B-294).

11 **Physiology**

COCHLEAR RESPONSES TO CONDENSATION AND RAREFACTION CLICKS. W.T. Peake and M.I.S. Kiang (intro. by M. Eden) Research Laboratory of Electronics, M.I.T.,Cambridge, Mass. and Eaton-Peabody Laboratory, Mass. Eye & Ear Infirmary, Boston, Mass.

Electrical responses to acoustic stimuli have been recorded from cats by gross electrodes located near the round window of the cochlea and in the internal auditory meatus. Neural responses to condensation and rarefaction clicks differ in certain aspects. The nature of the difference between these two responses is a function of the stimulus intensity. At low intensities the neural components are nearly identical. At high intensities the waveforms are similar, but the latency of rarefaction click responses is about 0.2 ms shorter than the latency of condensation click responses. At some intermediate intensities the responses differ strikingly in shape and size. These differences can be interpreted in terms of two excitatory mechanisms, which are operative in different intensity ranges. These mechanisms can be related to non-neural components of the cochlear response. These components have been studied in denervated preparations. The high intensity mechanism is related to the cochlear microphonic potential. The low intensity mechanism is related to a relatively slow potential which has the same polarity for both stimulus polarities. This slow component is prominent at low intensities. Supported by US Army (Signal Corp),USAF (OSR, ARDC) and USN (ONR).

12 **Physiology**

MAXWELL'S SPOT, HAIDINGER'S BRUSHES AND MACULAR PIGMENT. W.A.H. Rushton (intr.by M. Fuortes). Natl.Insts.of Health, Bethesda,Md.

It is 100 years since Maxwell saw his "spot" in blue light and attributed it to absorption by a pigment in front of the fovea, and 115 since Haidinger saw in blue polarized light his "brushes" which Helmholtz ascribed to absorption by yellow, doubly refracting fibres radiating from the foveal centre. Gullstrand denied presence of a yellow spot in the living eye and many have held it to be a ring round the fovea with a hollow just where pigment is needed to explain the phenomena of Maxwell and Haidinger. Physical measurements in the living eye can be made by the technique of retinal densitometry which permits comparison of reflectance from the eye in lights of two different wave lengths. The distribution of the pigment across the fovea was measured by comparing reflectivity in blue/orange light presented in 1° or ½° fields at various points on the retina. A localized yellow spot was found with maximum density at the foveal centre where Maxwell's spot is located. The spectral absorption of this pigment agreed with Wald's chloroform extract of macula and with Naylor & Stanworth's spectral sensitivity of Haidinger's brushes. Measurement of Haidinger's effect by retinal reflexion indicates that sensitivity to polarization is not caused by a "blue" visual pigment but by some retinal structure in front. Thus the interpretations of Maxwell and Helmholtz are confirmed.

13 **Physiology**

ANALYSIS OF CORTICOFUGAL INFLUENCES ON CUNEATE TRANSMISSION. Hardress J. Waller (intr. by V. E. Amassian.) Albert Einstein College of Medicine, New York.

Several workers have described centrifugal effects on somatosensory transmission (Hernandez-Peon, Dawson, Jabbur and Towe). Effects of repetitive stimulation of sensori-motor cortex on test responses of the medial lemniscal system were examined in locally anesthetized and paralyzed cats. Strengths of cortical stimulation sufficient to markedly reduce the test response to contralateral forepaw stimulation also produced a convulsive afterdischarge. Single cuneate units showed high-frequency discharge (e.g. 400-600/sec.) during and after cortical stimulation. Reduction of the test response to dorsal column stimulation remained after the cord was sectioned below at C2, which excluded activation of cuneate neurons by dorsal column relays. The cortical effect is predominantly crossed unless spileptic discharge spreads to the opposite cortex. The effect on the cuneate nucleus was reduced by more than one-half following section of the bulbar pyramid contralateral to the nucleus, implying that the pyramidal tract contributes significantly to the corticofugal action. These data probably do not relate to an attention mechanism, because synchronous activation of many corticofugal neurons is required to influence cuneate transmission. (Aided by USPHS grant NIH-B1603, C1.)

14 **Physiology**

DIAMETER AND RECEPTOR FUNCTION OF MYELINATED CUTANEOUS AFFERENT FIBERS. C.C. Hunt and A.K. McIntyre*. Univ. Utah College Med., Salt Lake City

Nearly 400 single afferent fibers from sural nerve have been studied by isolation in dorsal root filaments of cat. Conduction velocity was determined by measurement of latency after stimulus to intact sural nerve. Receptor characteristics related to each fiber were studied and extent of receptive field determined. The large majority of units could be characterized as touch or hair receptors. Touch receptor fibers had a unimodal diameter distribution principally in the range 7-14μ. Typically these had 1-4 very discrete receptive spots, were slowly adapting and often showed spontaneous discharge that was temperature-dependent in frequency. Rate and direction of temperature change as well as level were important in determining discharge frequency, most units being more responsive to cooling. Thus the same fibers may carry information as to touch and temperature. Hair receptor fibers showed a bimodal diameter distribution with modes at 3-4 and 10-11μ. They were rapidly adapting to varying degrees, varied in sensitivity, showed no baseline discharge and no effect of temperature. Occasional other receptors were noted, including some from structures beneath the skin. (Supported by a grant from US PHS.)

15 **Physiology**

RAT TASTE CELL PROLIFERATION. L. M. Beidler, M. S. Nejad*, R. L. Smallman*, and H. Tateda*. Florida State University, Tallahassee, Florida.

Colchicine arrests mitotic division in metaphase. Degeneration of rat taste cells in fungiform papillae is observed histologically within 4 hrs. after intraperitoneal injection of 1 cc of 0.1-0.5% colchicine/kg rat. Taste buds completely disappear within 10-20 hrs. Neural response of chorda tympani to chemical stimulation of tongue begins to decline within 3-4 hrs. after colchicine injection and completely disappears within 6-10 hrs. although tactile response remains. Spontaneous neural activity occurs during response decline and is absent after 8 hrs. Chromosomal nucleic acid was isotopicly labeled during mitotic division to locate dividing cells. Intraperitoneal injection of 0.7 μc tritiated thymidine/g rat labels DNA of taste cells within 4 hrs. Labeled taste cells are prominent in center of taste bud after 3-4 hrs. and spread to periphery of bud in 8 hrs. It is concluded that there exists a rapid turnover of cells in taste bud under normal conditions. The rapidity depends upon location of bud on tongue. This dynamic state necessitates continual reorientation of taste nerve endings. Functional changes with taste cell age are not yet known. (Supported by NSF Grant G3856.)

16 **Biochemistry**

PERMANENT FRACTIONATION OF THE ELECTRORETINOGRAM IN GLUTAMATE TREATED MICE. Albert M. Potts and Robert W. Modrell*. Univ. of Chicago, Chicago, Ill. and West. Reserve Univ., Cleveland,O.

It has been reported by others that a single sublethal dose of glutamate causes loss of ganglion cells and failure of development of bipolar cells in the retina of new born mice. After the fifteenth day of life such animals appear histologically to have a retina composed only of receptor cells plus one or two rows of glial cells. Electroretinograms of these animals show a large negative a-wave and no b-wave. This is a constant finding and is repeatable over many months. A logical conclusion of these findings is that only the a-wave is a direct reflection of retinal receptor cell activity. (Supported by a grant B-134 (now B-2522) from National Institute of Neurological Diseases and Blindness.)

197. NEUROPHYSIOLOGY– Spinal Cord

1 **Physiology**

GENERATION AND PROPAGATION OF THE SPIKE POTENTIAL IN SPINAL MOTONEURONS. C. A. Terzuolo and T. Araki *. Department of Anatomy, University of California at Los Angeles and Department of Physiology, University of Minnesota.

The result obtained with a double micro electrode which permits the penetration of a single motoneuron in two separated regions, or the simultaneous recording of the intra and extracellular potentials, will be presented. These results indicate: that the spike potential is generated peripherally to the initial segment; that the so-called A spike is due to the activity of a portion of the soma membrane; that the B spike is produced by the soma-dendritic membrane and propagates slowly out in the dendrites (where it has been recorded intracellularly); that a portion of the dendrite is not invaded by the spike. The problem of sink and source relationship between different portions of the membrane of motoneurons during the propagation of the spike potential will be discussed.

2 **Physiology**

ACTION OF TETANUS TOXIN ON RECURRENT CONDITIONING. V.J. Wilson, F.P.J. Diecke* and W.H. Talbot*. The Rockefeller Institute, New York City.

Tetanus toxin abolishes various types of reflex inhibition, leaving reflex excitation unaffected(Brooks et al.J.Physiol., 1957,135:655).Intraspinal toxin injections into the lumbosacral segments have been used to study the nature of recurrent facilitation and inhibition.Monosynaptic reflex size frequently increased following toxin injections or control injections not containing toxin.Therefore,not only maximal reflexes but also sub-maximal reflexes,kept at fairly uniform size throughout the experiment,were used as test reflexes.Control injections had little effect on recurrent conditioning. Tetanus toxin consistently abolished,or strongly depressed, both recurrent inhibition and recurrent facilitation of a given nucleus(similar results have been observed with i.v. injections of strychnine).In contrast,orthodromic polysynaptic facilitation was unaffected or increased in similar experiments. Tetanus toxin thus distinguishes between orthodromic and recurrent facilitation.Abolition of the latter strongly suggests that inhibitory junctions are involved in the pathway for recurrent facilitation.It is suggested that recurrent facilitation is a disinhibition,which enhances reflex discharge by the removal of tonic inhibitory activity.

3 **Physiology**

BILATERAL FACILATATORY AND INHIBITORY SKIN AREAS OF SPINAL MOTONEURONES OF THE CAT. D. Megirian (intr. by A. L. Towe.) Department of Physiology & Biophysics, University of Washington, Seattle, Washington.

In spinal cats monosynaptic reflex (MR) discharges were recorded from single motor axons (ventral roots L_6, L_7 or S_1) supplying knee and ankle flexors and extensors. The influence of nociceptive cutaneous stimulation on the probability of a test MR, evoked continuously at 10-25/sec., was determined, and the skin fields were mapped as facilatatory (F) or inhibitory (I). The boundaries between F and I areas were not sharp but overlapped somewhat. The size of the F and I fields for individual members of a muscle group, e.g. ankle flexors, sometimes varied widely. For knee extensors variation was minimal, and the motoneurones could be identified by their F and I maps. Bilateral conditioning effects were most pronounced when the skin areas lay over the digital and ankle myotatic units when test MR was evoked from the latter. In general, nociceptive stimulation induces bilateral flexion (dorso-lateral surface) or extension (ventromedial surface) when the skin area is proximal and ipsilateral flexion and contralateral extension when the skin area is distal. (Supported by PHS Spec. Traineeship BT-402 and grant 2-B5082 from the National Institute of Neurological Diseases & Blindness.)

4 **Physiology**

INTRACELLULAR POTENTIALS FROM DORSAL ROOT GANGLION OF CAT. Makoto Sato* and George Austin, Division of Neurosurgery, University of Oregon Medical School, Portland, Ore.

Intracellular recordings have been made from L5 & L6 ganglia of 51 unanesthetized decerebrate cats. Dorsal roots were cut centrally to avoid dorsal root reflex activity. Adequate stimuli such as tactile and pressure were adopted to skin, hair, muscle and tendon of the hind limb and paw. Initial amplitude of membrane potentials varied from 20 to 80 MV and action potentials from 20 to 65 MV, rarely showing overshoot. Dorsal root ganglion cell potentials were interpreted as differing from fiber potentials by the following points; 1) The former can be recorded through at least a 50μ range, and within 200μ in depth from surface. 2) The former show a slower response with larger after-potential. 3) Membrane potentials of the former are larger, much stabler, (lasting more than 20 minutes occasionally), and show longer refractoriness. In response to natural stimulation 3 different patterns were seen; 1) Those firing only in response to stimulation. 2) Those showing spontaneous repetitive activity that could be facilitated by stimulation from one area and inhibited from another. Electrostimulation of dorsal root was also made to compare the evoked potential of non-dendrite cells with that of dendritic cells (motoneurons stimulated antidromically).

5 **Physiology**

SINGLE SPINAL MOTONEURON EXTRACELLULAR POTENTIAL FIELDS. P. G. Nelson*, K. Frank and W. Rall. Natl. Insts. of Health, Bethesda, Md.

Such extracellular fields were studied by Fatt (J. Neurophysiol., 1957). To further ensure that only one motoneuron produces the recorded potentials, a technique for stimulation and recording from single ventral root fibers was developed in the present study. Also, the fields associated with the A spike and AB spike (Fuortes, Frank and Becker, J. Gen. Physiol., 1957) were both recorded. Potential measurements based on one cell showed: (1) The field of the AB spike can be recorded above noise level in a region extending 500-600μ dorsoventrally, more than 300μ cephalocaudally, and more than 200μ mediolaterally. (2) The A spike field extends less widely, i.e., 300μ dorsoventrally, 200μ cephalocaudally and 100μ mediolaterally. (3) In the periphery of its field the AB spike is positive-negative; in a relatively restricted (100μ diameter) central region it is positive-negative-positive. (4) The A spike alone is pure negative in a restricted region, and is positive-negative at other parts of its field. (5) Similar small variations in latency were observed for A and AB fields. These fields are being subjected to theoretical analysis.

XUM

6 *Physiology*

REFLEXES IN SPINAL CORDS WHICH HAVE RECOVERED FROM ASPHYXIATION. A. van Harreveld and D. Spinelli. California Institute of Technology, Pasadena, California.

Spinal cords which have recovered for 2 weeks from 40-50 minutes of complete asphyxiation show no obvious reflex activity, except perhaps some tone. Stimulation of dorsal roots causes reflex action potentials in the ventral roots, however. This activity has the following features. The post-activation depression is considerably less marked than in normal preparations and is of relatively short duration (about 100 msec). Inhibition of reflex action potentials by preceding antidromic volleys (Renshaw inhibition) is usually completely lost. The reflex action potentials can be elicited at frequencies of 100 to 200/sec. Facilitation and post-tetanic potentiation are only indicated. Part of the features of these spinal cords can be explained by the loss of small (internuncial) cells which are damaged more severely by asphyxiation than the motoneurones (e.g. the loss of Renshaw inhibition). The slightness of the facilitation and potentiation observed in these preparations does not follow directly from the loss of internuncial cells, but could be due to an increased excitability of the remaining motoneurones. (Supported by a grant from the Office of Naval Research, Contract: Nonr 220, 34).

7 *Pharmacology*

COMPARATIVE NEUROPHARMACOLOGY OF FOUR INTERNEURONAL DEPRESSANTS. P. Spitaletta, L. B. Witkin, F. Galdi and M. Maggio (intr. by A. J. Plummer) Research Dept., CIBA Pharmaceutical Products Inc., Summit, N. J.

Previous experiments have shown that mephenesin has an effect in cats (C-1 transection) of selectively depressing spinal polysynaptic pathways in doses which leave monosynaptic reflexes such as the patellar reflex intact. Similar experiments performed in intact cats under chloralose-urethane confirm the above findings and have been extended to indicate that this effect of mephenesin is specific for compounds with this action in that a variety of other central depressants including phenothiazines and hypnotics (barbiturate and non-barbiturate) cause a non selective depression of both reflexes. Mephenesin further has the ability of blocking the pinna reflex of the mouse in doses below those which block the corneal reflex. Styramate, chlorzoxazone and methocarbamol have been compared to mephenesin for their effects on cat spinal reflexes and studied in mice in the following tests: pinna reflex, corneal reflex, righting reflex, acute toxicity, spontaneous motor activity (photoelectric activity cage), and anticonvulsant activity as well as their comparative duration of action.

198. NUCLEIC ACIDS

1 *Biochemistry*

NUCLEIC ACIDS AND INDUCED AMPHIBIAN METAMORPHOSIS. Frank J. Finamore* and Earl Frieden, Department of Chemistry, Florida State University, Tallahassee, Florida.

The metabolic activity of liver and tail nucleic acids, as measured by the incorporation of P^{32}-orthophosphate was studied to correlate morphological and enzymic changes with nucleic acid metabolism during induced metamorphosis. Rana grylio tadpoles were injected i.p. with 1×10^{-6} moles/g. body weight of 3,5,3'-triiodothyronine to induce metamorphosis, followed one hour later by 100 μc of carrier-free P^{32}. Control animals received only an equivalent amount of the isotope and at various times after injection both control and hormone-treated tadpoles were sacrificed. During induced metamorphosis and incorporation of P^{32} into RNA, DNA, and protein of the liver increases and is observable before any external morphological changes. There is a marked alteration of the liver RNA/DNA during this time due to a net synthesis of RNA coinciding with an apparent liver hypertrophy. Incorporation of P^{32} into the RNA, DNA, and protein of the tail increases for two days after initiation of metamorphosis, then is maintained at a relatively constant level. In contrast, the specific activities of the control group continue to increase. These results suggest the premature establishment of an apparent "steady state" in the tail during induced metamorphosis. (Supported by USPH Grant 3006.)

2 *Biochemistry*

STUDIES ON A DNA PHOSPHODIESTERASE FROM E. COLI. I. R. Lehman (intr. by R. L. Baldwin). Stanford University School of Medicine, Palo Alto, California.

A phosphodiesterase has been purified about 150 fold from extracts of E. coli which has the following properties:

(1) It shows only slight activity with intact, double-stranded DNA, but rapidly hydrolyzes heated, double-stranded DNA or DNA from bacteriophage ΦX 174 to 5' mononucleotides. Hydrolysis proceeds in a stepwise manner beginning at the 3' hydroxyl end of the chain.

(2) The enzyme is unable to cleave free dinucleotides or the 5'-terminal dinucleotide of a polydeoxynucleotide chain. As a result, its action on a pancreatic DNase limit digest of calf thymus DNA in which the average chain length is 4, produces a digest consisting of 1/2 mononucleotides and 1/2 dinucleotides. In a polydeoxynucleotide chain several thousand nucleotides long, the 5'-terminal dinucleotide represents a negligible proportion of the total, hence most of the chain is hydrolyzed to mononucleotides.

(3) In contrast to venom diesterase, this enzyme is able to degrade bacteriophage DNA's bearing glucosylated hydroxymethyl cytosine to their constituent mononucleotides.

3 *Biochemistry*

COMPARISON OF THE BIOLOGICAL EFFECTS OF 5-CHLORODEOXYURIDINE WITH OTHER 5-HALOPYRIMIDINE DEOXYRIBOSIDES. David M. Frisch*, Bessie Huang*, and Donald W. Visser. Dept. of Biochem. and Nutrition, Univ. of Southern California, School of Med., Los Angeles, Calif.

5-Chlorodeoxyuridine (CldU) was synthesized for the purpose of comparing its inhibitory properties with 5-fluorodeoxyuridine (FdU) and 5-bromodeoxyuridine (BrdU). Using Escherichia coli K-12 as the test organism it was found that FdU was 4,000 times more effective than CldU which in turn was 9 times more effective than BrdU for inhibiting growth of the bacteria to 50 percent of a control. CldU inhibited growth by mechanisms similar to that of BrdU. Low concentrations of CldU and BrdU potentiate the inhibitory effect of FdU by limiting the utilization of thymidylic acid for DNA synthesis. Higher concentrations of CldU or BrdU serve as thymidine substitutes for DNA synthesis. In preliminary studies, germinating rye grass seeds were used to determine the effects of 5-bromodeoxycytidine, 5-chlorodeoxycytidine, FdU, BrdU and CldU on root and blade growth. All of these compounds primarily inhibited root growth. FdU was the only derivative which inhibited growth completely. Thymidine completely or partially reversed the inhibitory effects of these derivatives.

4 *Biochemistry*

COMPOSITION OF METABOLICALLY ACTIVE RNA IN PHAGE-INFECTED BACTERIA. L. Astrachan (intr. by W. E. Cohn). Oak Ridge Natl. Lab., Oak Ridge, Tenn.

Investigations of RNA metabolism in phage-infected E. coli have indicated that shortly after infection a new RNA is synthesized and that the composition of this RNA resembles DNA of the infecting phage. Evidence for new RNA is derived from alkaline hydrolysis of P^{32}-labeled RNA followed by separation of the resultant 2',3'-nucleotides. These had specific radioactivities that varied exactly in proportion to the composition of DNA in the infecting phage. If the data are correctly interpreted to indicate synthesis of a new RNA, the distribution of specific activities found when RNA is hydrolyzed to 5'-nucleotides should also resemble the phage DNA. Although it is found that this distribution changes with time after P^{32} addition, it reaches, within 20 minutes, the distribution found at all times with 2',3'-nucleotides. This indicates that RNA is synthesized from 5'-nucleotide precursors which do not have equal specific activities immediately after P^{32} addition. Alkaline hydrolysis, however, serves to equalize the specific activities of the precursor pool so that the RNA composition may be calculated from the relative specific activities of the 2',3'-nucleotides.

5 *Biochemistry*

BIOLOGIC ACTIVITY OF RNA IN T2 INFECTION. Elliot Volkin. Biology Division, Oak Ridge Natl. Lab., Oak Ridge, Tenn.

To test for biological function of RNA synthesized in E. coli B after infection by T2, we utilized the mutant B94, kindly made available to us by Dr. Joseph Gots. The mutant, which requires both adenine and arginine for growth, will not support production of T2 in the absence of either adenine or arginine; in addition, neither T2 synthesis nor bacterial growth occurs in the presence of deoxyadenosine plus arginine. Washed cells infected with T2 in synthetic medium containing adenine alone synthesize some RNA specific to the T2 system in the absence of DNA or protein synthesis. After accumulating such RNA for about an hour, the cells are washed free of adenine, incubated in minimal medium to exhaust any endogenous adenine, then given arginine and deoxyadenosine. Production of T2 bacteriophage will now ensue in this latter medium that is incapable of supporting phage synthesis without prior formation of the RNA.

6 *Immunology*

STUDIES OF A BACTERIAL RIBONUCLEIC ACID INHIBITOR OF STREPTOCOCCAL DNASE B AND STREPTOCOCCAL RNASE ACTIVITIES. By Lewis W. Wannamaker, Univ. of Minnesota, Minneapolis, Minn.

Previous studies have indicated that group A streptococci produce three DNases (A, B, and C) which can be separated by starch zone electrophoresis, are immunologically distinct, and show differences in inhibition by citrate and in optimal pH of activity (J.Exp.Med. 107:797, 1958). In searching for further biological differences in the three DNases, the effect of the bacterial RNA inhibitor of Bernheimer (Biochem.J. 53:53, 1953) has been studied. A partially purified preparation of this inhibitor (ℓ(P)=8300, atomic N/P = 3.9, protein = 5%, DNA = 8%) was obtained from streptococci. In a final concentration of 5 µg/ml, this preparation inhibited the depolymerization of calf thymus DNA (1 mg/ml) by DNase B (35 units/ml, final conc.) approx. 70% as measured viscosimetrically, whereas concentrations of 200 µg/ml failed to inhibit comparable amounts of DNase A or DNase C. The biological activity of the inhibitor was destroyed by pancreatic RNase but was not affected by pancreatic DNase, by streptococcal DNases A, B, or C, nor by streptococcal RNase. Moreover, the depolymerization of yeast RNA by streptococcal RNase, as assayed turbidimetrically, was markedly reduced in the presence of this inhibitor. These findings suggest two alternate possibilities: (1) the two activities are associated with two distinct enzymes both of which are inhibited by bacterial RNA or (2) the two activities are related to a single nuclease which depolymerizes both calf thymus DNA and yeast RNA but is inhibited by bacterial RNA. Unsuccessful attempts to separate streptococcal DNase B and streptococcal RNase activities by zone electrophoresis and by column chromatography support the latter possibility.

199. NUCLEIC ACIDS–DNA

1 *Physiology*

PROTEASE ACTIVITY OF HISTONES IN DEOXYRIBONUCLEOPROTEIN. Arthur J. Emery, Jr. and Stephen A. Lesko, Jr. (intr. by G. G. Rudolph.) Univ. of Md. Sch. of Med., Baltimore, Md.

The ability of deoxyribonucleoprotein (DNA-P) to form a recoilable gel in molar saline or alkaline solution has been used as a criterion that DNA is attached firmly to the residual protein. DNA-P prepared from calf thymus by the method of Doty does not form a recoilable gel whereas the DNA-P prepared by the method of Dounce is capable of forming this gel. Conditions for the isolation of DNA-P by the method of Doty apparently inhibit the cellular nucleases, however the conditions are almost optimal for the activity of proteases. The action of proteases could account to a large extent for the loss of the ability of DNA-P to form a gel when prepared by Doty's method. The present study has shown that DNA-P prepared from calf thymus and rat liver by the methods of Doty and Dounce exhibit chymotrypsin and trypsin activity. All of the chymotrypsin and none of the trypsin activity was found in the histone that could be extracted from the DNA-P by 0.2N HCl. Additional histone extracted by 1M NaCl, pH3,revealed that some of the trypsin activity could be extracted whereas the rest of this activity remained in the residual protein of the extracted DNA-P. Only the proteases in the DNA-P prepared by Doty's method were apparently active during the isolation procedure. (Supported by USPHS Grant No. RG-5874.)

2 *Biochemistry*

REPLICATION OF SEQUENCES IN THE ENZYMATIC SYNTHESIS OF DNA. John Josse* and Arthur Kornberg. Stanford University School of Medicine, PaloAlto, California.

DNA was enzymatically synthesized with P^{32}-labeled substrates and then degraded to 3'-deoxymononucleotides by specific DNases. The amounts of each of the 16 possible dinucleotide (nearest neighbor) sequences have been determined using as primer DNA, samples from 6 different natural sources. The conclusions are: 1) All 16 possible dinucleotide sequences are found in each case. 2) The pattern of relative frequencies of the sequences is unique and reproducible in each case and is not predicted by the base composition of the DNA, and 3) Enzymatic replication involves base pairing of A to T and G to C in 2 strands running in opposite direction as predicted by the Watson and Crick Model.

3 *Biochemistry*

PRIMERS FOR CALF THYMUS POLYMERASE. F. J. Bollum (intr. by C. W. Carr). Biology Division, Oak Ridge Natl. Lab., Oak Ridge, Tenn.

Calf thymus polymerase will not utilize native DNA as primer for DNA synthesis. Deoxypolynucleotide suitable for primer may be formed from native DNA by any physical or chemical treatment which leads to denaturation of the double-stranded molecules. Addition of pancreatic DNase directly to reaction mixtures containing native DNA results in primer formation in situ due to denaturation by single-chain scission. DNA digested by micrococcal nuclease or venom diesterase will not serve as primer unless it is denatured after digestion. Sonic degradation of native DNA does not lead to primer formation, the sonic fragments also requiring denaturation before becoming primers. All of these results suggest that the release of H-bonds is a prerequisite for priming action and that single strands or single-stranded regions are the actual priming site for deoxypolynucleotide synthesis by calf thymus polymerase. Experimental confirmation of this postulate is provided by (1) ØX-174 DNA (generously provided by R. L. Sinsheimer) which primes without heating, (2) the observation that acid-soluble deoxypolynucleotides from a pancreatic DNase digest are primers, and (3) preliminary observations on the synthetic polymers pTpTpT and pTpTpTpT (kindly furnished by H. G. Khorana) which indicates formation of polymers from dATP alone.

4 *Biochemistry*

THE REACTION OF DNA WITH THIOLATING REAGENTS. Edward L. Duggan, Stuart L. Bunch* and William D. Moriarty*. Dept. of Biochemistry, Univ. of California School of Med., San Francisco.

In order to determine the reactivity of amino groups in denatured and undenatured DNA, attempts were made to react thiolating reagents with various preparations. The DNA was isolated from chicken erythrocytes by the dodecyl-sulfate method of Stevens and Duggan (1958) which originated from a similar one of N. S. Simmons (1955). The thiolating reagents used were those of Benesch (1956) and Klotz (1959). Both of these reagents, N-acetyl homocysteine thiolactone and acetyl succinic anhydride were reported to react with protein amino groups with the liberation of one sulfhydryl for every amino group reacted. Analysis of sulfhydryl was by the method of Roberts and Rouser (1958) and of Alexander (1958), i.e. by the decrease in absorbance of N-ethyl maleimide at 300 mu. Various reaction conditions were tried: pH 4-12 and 0-100°. Kinetic studies revealed that no reaction occurs with N-ethyl maleimide, even with Ag^+ as a catalyst. Some denaturants, notably guanidine HCl, were found to cause "degradation" of DNA to dialysable components. (Supported by grants from the American Cancer Society and USPHS.).

XUM

5 *Biochemistry*

DEOXYRIBONUCLEIC ACID POLYMERASE ACTIVITY IN EXTRACTS OF NOVIKOFF HEPATOMA TISSUE. N. Burr Furlong* and A. C. Griffin The Univ. of Texas M. D. Anderson Hosp. and Tumor Inst., Houston, Texas.

The soluble fraction obtained from the ultracentrifugation of Novikoff hepatoma homogenate has been found to possess DNA polymerase-like activity. Incubation of the soluble fraction with the triphosphates of deoxyadenosine, deoxyguanosine, deoxycytidine and thymidine-2-C^{14} in a medium also fortified with ATP, $Mg\ Cl_2$, and DNA primer resulted in incorporation of 20% of the C^{14} into the acid insoluble portion of the incubating system. Treatment of the labeled insoluble portion with DNase for one hour at pH 7.5 resulted in 80% of the C^{14} appearing in the supernatant liquid upon reacidification. A 20-fold increase in specific activity was obtained by allowing the soluble tumor extract to percolate through DEAE cellulose. Approximately 99% of the total protein is adsorbed by this treatment; however a substantial but variable portion of activity is found in the sample effluent. (Supported by a Rosalie B. Hite Fellowship and a grant from the American Cancer Society.)

6 *Biochemistry*

FRACTIONATION OF ANALOGUE-CONTAINING TRANSFORMING PRINCIPLE BY CENTRIFUGATION IN THE CsCl GRADIENT. W. Szybalski, Z. Opara-Kubińska, and E. Ephrati-Elizur. (intr. by M. Heidelberger) Inst. of Microb., Rutgers Univ., New Brunswick, N.J., and Dept. of Biochem., Columbia Univ., New York, N.Y.

5-Bromodeoxyuridine (BUDR) is incorporated into the DNA of Bacillus subtilis during growth in the presence of this analogue (Ephrati-Elizur and Zamenhof, Nature 184:472, 1959). DNA was extracted from lysed cells, deproteinized, and freed of RNA by RNase treatment. When centrifuged for 72 hrs at 35,000 r.p.m. in the Spinco swinging bucket rotor, the BUDR-free ("light") DNA molecules converged to a position in the CsCl gradient corresponding to a density of approx. 1.702, forming a narrow band. Density, DNA content and transforming activity were determined in all fractions collected by the method described by Szybalski (Experientia, in press). The BUDR-containing DNA banded predominantly at density 1.750 ("heavy") with progressively smaller, broad peaks formed at densities corresponding to "semi-heavy" and "light" DNA. The transforming activity (indole marker) of the isolated fractions is correlated with their DNA content. It may be concluded that the DNA molecule is biologically functional, even after extensive substitution of BUDR for thymidine in one or both strands. (Supported by grants from the National Science Foundation, G-7021 to W. Szybalski and G-4337 to S. Zamenhof).

7 *Biochemistry*

MOLECULAR WEIGHT AND AGGREGATION OF DNA. Liebe F. Cavalieri and Joan Deutsch*. Sloan-Kettering Inst. for Cancer Research, New York 21, N. Y.

In recent years it has appeared that the molecular weight of DNA centered around the value of 6×10^6 with occasional values of two to three times this. Recent work in this laboratory shows the range to be $1\text{-}2 \times 10^6$ as determined by light scattering; the higher molecular weights represent aggregates, arising perhaps as artifacts. DNA isolated from a number of sources has been disaggregated by treatment with chymotrypsin or shaking with chloroform-octanol without denaturation. The decrease in molecular weight is accompanied by a greater extension of the molecule; the association appears to be primarily lateral. Samples from sources such as E. coli and calf thymus react vastly differently toward the various disaggregation procedures. The success of the chloroform-octanol procedure depends both on the ionic strength and pH of the medium. The molecular weight of calf thymus DNA disaggregated in this manner is then pH dependent. It has also been found that aggregation of calf thymus DNA can be readily induced by close-packing of the molecules such as in a pellet obtained from high-speed centrifugation.

8 *Biochemistry*

CONSECUTIVE DIGESTION OF DNA BY TWO DNASES. S. Vanecko* and M. Laskowski. Dept. of Biochemistry, Marquette Univ. School of Medicine, Milwaukee, Wis.

Thymus DNA was first digested with splenic DNase II and the products were chromatographed on Dowex-1-X8. The fraction eluted with 2 M ammonium acetate buffer, pH 4.5, was collected, lyophilized and used as substrate for pancreatic DNase I. The products of the second digestion were chromatographed and smaller fragments were identified. Neither nucleosides nor mononucleotides were detected, but some mononucleoside-3',5' diphosphates were present. Dinucleoside monophosphates were abundant and numerous but the combination PupPy was absent. In this fraction the combination CpX predominated over TpX, and total purines over total pyrimidines. Dinucleotides were also present and some were identified. The complexity of the mixture of fragments excludes the assumption that some internucleotide linkages are totally resistant to both DNases. The preferential susceptibility of a particular linkage is confirmed and appears to be governed by the length of the chain, the adjacent bases, and the proximity of the free phosphoryl group. (Supported by the grants of the Atomic Energy Commission and the American Cancer Society.)

9 *Biochemistry*

PROTEASES OF CALF THYMUS NUCLEI. N. K. Sarkar* and A. L. Dounce, University of Rochester School of Medicine and Dentistry, Rochester, N. Y.

It has been found that failure of the thymus DNA-nucleoprotein of Zubay and Doty to form gels can be caused either by the high speed of the Waring blendor or by protease action or both. The importance of blendor speed is shown by the fact that DNA itself can be denatured by high-speed stirring in a blendor. Protease action is indicated by the occurrence of slow degelation even when blendor speed has been greatly reduced. Thymus nuclei isolated in 0.9% NaCl were tested for hydrolytic action against carbobenzoxy-L-glutamyl-L-tryosine at pH 3.8 to 4.5, N-acetyl-L-tyrosine ethyl ester at pH 6.0 to 9.0, and benzyl-L-arginine methyl ester at pH 7.0 to 9.0. Fairly strong hydrolysis was noted in the first two cases at certain pH values indicating cathepsins A and C, and weak hydrolysis in the last case indicating cathepsin B. The nucleoprotein of Zubay and Doty contained all three of the above-mentioned enzymes. A suspension of calf thymus nuclei undergoes auto-proteolysis in one week at $4^{\circ}C$. so that neither trypsin nor chymotrypsin will liberate more tyrosine and/or phenylalanine, and gelability is concomitantly lost. These data indicate that proteolysis as well as blendor action may be a factor causing the failure of the nucleoprotein of Zubay and Doty to form gels.

10 *Biochemistry*

STUDIES ON THE REPLICATION OF COMPONENTS OF NUCLEOHISTONES. F. R. Frankel*, J. Knapp* and C. F. Crampton. Depts. of Pathology and Biochemistry, College of Med., Univ. of Florida, Gainesville, Florida.

Molecules of DNA separable by use of columns of Mg IRC-50 (Fed. Proc., 17, 206) may be synthesized at different rates, or from pools of precursors that become labeled at different rates, because a 7 fold difference has been found in the specific activity of thymine among fractions from single specimens of DNA isolated from 5 day cultures of Ehrlich ascites tumor cells removed from mice 5 min. after the i.p. injection of C^{14}-formate or H^3-thymidine. There is no difference in the specific activity of thymine among fractions of DNA when the cells are removed 1 day after injection. When 2-C^{14}-glycine or a mixture of H^3-thymidine plus 1-C^{14}-leucine are injected 1 to 4 days prior to harvesting, the ratios of cpm/mg of DNA to cpm/mg of histone Fraction A (J. Biol. Chem., 225, 363) or arginine-rich histone are 1.1 to 1.3 times greater in 4 day cultures of the cells than in 5 day cultures. Since the doubling time of these cells is about 18 hr., it would appear that the substance of the histones is conserved at least as well as the DNA in this system. However, the results thus far do not indicate whether the histones persist intact and remain combined with DNA during replication. (PHS RG-4801 made these studies possible.)

11 *Biochemistry*

THE BIOLOGICAL ACTIVITY OF THERMALLY DENATURED TRANSFORMING DNA. J. Marmur and D. Lane. Harvard University, Cambridge, Mass.

When DNA isolated from streptomycin resistant Diplococcus pneumoniae is heated in solution at a temperature where the molecules are believed to be denatured as measured by the optical density increase at 260 mμ and then cooled, varying amounts of transforming activity remain. This residual activity is probably due to the partial reformation of complementary strands into helical regions which are necessary to satisfy one or more steps in the transformation process e.g. adsorption to host cells. The biological activity is dependent upon the conditions of the denaturation and the subsequent cooling. Thus denatured DNA which exhibits 0.2% residual transforming activity as the result of rapid quenching to 0° at low ionic strength and low DNA concentration will possess up to 30 times higher activity when cooled slowly at high ionic strength and high (20 ug/ml.) DNA concentration. Evidence that the two strands of the DNA molecules separate and partially reform in a specific manner during the thermal denaturation and cooling is demonstrated by showing that the addition of an excess of denatured homologous (but not of heterologous) wild type DNA during the cooling increases the biological activity by a factor of two. This suggests that one of the two strands is required to carry the information to transform to streptomycin resistance. (supported by a Public Health Service Grant [C-2170]).

12 *Biochemistry*

THE REACTION OF PURINES, PYRIMIDINES AND DNA WITH FORMALDEHYDE. S. Levine*, L. Grossman, and M. Simon*. Graduate Dept. of Biochemistry, Brandeis Univ., Waltham, Mass.

Formaldehyde reacts with amino-containing purine and pyrimidine nucleotides causing bathochromic and hyperchromic shifts in their U.V. spectra (Fraenkel-Conrat, BBA 15, 307, 1954). These effects have been observed with AMP, GMP, and CMP but not with UMP, TMP, or IMP. Reactivity with formaldehyde, judged by these spectral changes, is pH and temperature dependent. I.R. spectra show that formaldehyde reacts preferentially with the amino group causing a marked diminution and slight shift in the amino vibrational frequency at 1626 $cm.^{-1}$. Application of this reaction to native and heat-denatured DNA indicates that the response to formaldehyde parallels that observed with the nucleotides. Such a response is observed only with the heat-denatured DNA. Dialysis of this material results in restoration of the spectrum to that observed with the original heat-denatured DNA indicating the reversibility of the formaldehyde reaction. C^{14}-formaldehyde is not fixed in heat-denatured DNA due to this unfavorable equilibrium. The reactivity of purine and pyrimidine amino groups with imido esters is presently being investigated and will be discussed. (Supported by grants from NSF and NIH).

13 *Biochemistry*

INCORPORATION OF RADIOACTIVE DNA INTO EHRLICH ASCITES CELLS M. R. Chorazy, H. H. Baldwin and R. K. Boutwell (intr. by E. C. Miller) McArdle Mem. Lab., Univ. of Wis., Madison.

Although genetic transformation by exposure to DNA has been demonstrated in micro-organisms, it has not been shown unequivocally in mammalian systems. Evidence for the absorption of intact DNA by mammalian cells would establish the necessary condition for subsequent biological effect. Ehrlich ascites cells were incubated in Robinson's medium containing DNA-H^3. (The labelled DNA, $6x10^6$ to $3.5x10^7$ cpm per mg. DNA, was isolated by salt extraction from Ehrlich ascites cells incubated with tritiated thymidine.) After incubation the cells were separated from the medium and radioactivity was determined in the acid soluble and DNA fractions of the medium as well as the acid soluble and the DNA fractions of the cells. Absorption of DNA by the cells began immediately and continued for 2 hours. There was no change in the specific activity of the DNA of cells incubated with amounts varying between 0.05 and 0.8 mg. of DNA-H^3 per flask. Incubation of cells in a medium containing a constant concentration of DNA-H^3 and increasing amounts of non-radioactive thymidine (0.1, 1.0, and 10 mM per flask) did not lower the specific activity of cell DNA. The data show that Ehrlich ascites cells are capable of absorbing at least large fragments of DNA molecules under in vitro conditions.

14 *Biochemistry*

ENZYMATIC SYNTHESIS OF A COPOLYMER OF DEOXYADENYLATE AND DEOXYTHYMIDYLATE. C. M. Radding*, J. Adler*, and H. K. Schachman. Biochem. Depts., Stanford Univ. and Univ. Calif., Berkeley.

Polymerase from E. coli catalyzes the synthesis of a copolymer containing dAMP and dTMP in alternating sequence. Unlike the enzymatic synthesis of DNA, this new polymer (d-AT) is formed de novo in the absence of demonstrable primer. Of the deoxynucleoside triphosphates present 80% are converted to polymer. This conversion was studied by the (1) increase in viscosity, (2) decrease in A_{260}, and (3) incorporation of labeled substrates into an acid insoluble product. When dGP^{32}PP or dCP^{32}PP is added to the reaction mixture the polymer produced contains less than 0.03% of the radioactivity. 5BrdUTP substitutes for dTTP leading to the formation of a large polymer. Synthesis of d-AT is exponential after a lag period of 3 hours. The lag period is related inversely to the enzyme concentration and is eliminated by enzymatically synthesized d-AT. The polymer which is hypochromic and melts sharply and reversibly at 71°C has a sedimentation coefficient of 20S and an intrinsic viscosity of 40 $(g/100\ ml)^{-1}$ corresponding to a molecular weight of about 5×10^6. These physical properties indicate stiff and elongated macromolecules similar to doubly stranded DNA molecules. The size of the product is related directly to the duration of the lag period. Studies of the mechanism of de novo and primed syntheses will be reported.

15 *Biochemistry*

IODODEOXYURIDINE (IDU) AS A TRACER OF DNA METABOLISM IN VIVO. R. C. Krueger*, D. Gitlin*, S. L. Commerford*, J. Stein* and W. L. Hughes. Brookhaven National Laboratory, Upton, N.Y. and Children's Medical Center, Boston, Mass.

Mice maintained on NaI in drinking water were injected with trace amounts of IDU labeled with I-131 (less than 0.1 μM per mouse). Within one hour following injection essentially all of the radioactivity was converted to iodide or incorporated into DNA. As radioiodide is rapidly excreted, the residual radioactivity after 24 hours was all found in DNA. At this time the concentration of radioactivity in several organs varied 50-fold and decreased in the order: thymus, gut, spleen, skin, bones, liver, muscle, in accord with their proliferative activity. The radioactivity of all organs except skin declined during the following days. This decline presumably measures cell turnover since the skin radioactivity remained constant throughout the period of observation (one week). Double tracer experiments with both IDU and H^3-thymidine showed thymidine to be utilized 3-4 times as efficiently as IDU. The I-131 radioactivity of the DNA of intestine declined more rapidly than H^3 activity (from H^3-thymidine) during cell turnover indicating that thymidine is measurably reutilized in this process. (Supported by the U. S. Atomic Energy Commission and the National Institutes of Health.)

16 *Biochemistry*

THE INCORPORATION OF RIBONUCLEOTIDES AND DEOXYRIBONUCLEOTIDES INTO FRACTIONS OF CALF THYMUS NUCLEI. Joseph S. Krakow and H.O.Kammen (intr. by G.R.Wyatt) Dep't of Pharmacology, Yale University Med. School, New Haven, Conn.

Disruption of nuclei isolated from calf thymus has yielded a fraction capable of incorporating ribonucleotides and deoxyribonucleotides into non-dialyzable acid-insoluble material. The incorporation of both types of nucleotides exhibits maximal activity at pH 7.0-7.5, requires Mg^{++}, and ATP (or an ATP generating system), and is inhibited by pyrophosphate. The incorporation of both types of nucleotides is not appreciably inhibited by RNase, but is strongly inhibited by DNase. The radioactivity in the product labeled with C^{14}-CTP is stable to alkaline hydrolysis, but becomes acid-soluble upon treatment with pancreatic DNase. Treatment of the C^{14}-labeled product with micrococcal DNase and spleen diesterase indicates that a large percentage of the incorporated radioactivity is in the form of terminal cytidylic acid. The incorporation of C^{14}-dGMP is stimulated by the addition of the complementary deoxyribonucleotides; the addition of the corresponding ribonucleotides is without effect.

17 *Pathology*

INCORPORATION OF INTACT HETEROLOGOUS DNA INTO STRAIN L FIBROBLASTS. Donald W. King and Klaus G. Bensch* Yale Univ. Sch. of Med., New Haven, Conn.

Although the transformation phenomenon is well known in bacteria, particularly the pneumococci , similar experiments with mammalian cells have shown little success. Part of the problem has been to incorporate DNA in an intact form. We have extracted DNA from Harding-Passey mouse melanoblasts and sarcoma 180 by the method of Zamenhof (Methods in Enzymology, vol. III, p. 697, Academic Press, 1957). DNA particles coated with gelatin have been found to be readily phagocytized by strain L fibroblasts in suspension tissue culture. The DNA in the cytoplasm has been examined by means of the Feulgen stain as well as with radioautographs made after incubation with nucleic acid particles labeled with tritiated thymidine.

200. NUCLEIC ACIDS–Nucleotides

1 *Biochemistry*

ENZYMATIC REDUCTION OF 5'-CYTIDYLIC ACID TO DEOXYCYTIDYLIC ACID E. C. Moore (intro. by G. A. LePage.) The Univ. of Texas M. D. Anderson Hosp. and Tumor Inst., Houston, Texas.

The reduction of CMP-C^{14} to deoxycytidine nucleotides (isolated chromatographically with carrier dCMP) has been demonstrated in a cell-free extract of rat tumor. Yields of up to 0.03 μmole of dCMP were obtained by incubating 0.4 μmole of CMP-C^{14} for 2 hours with 1 ml of dialyzed supernatant from the ultracentrifugation of a 40% homogenate of Novikoff ascites hepatoma cells, in Tris buffer supplemented with ATP, $MgCl_2$, and TPNH. Cytidine-C^{14} was rapidly phosphorylated by the extract and was as good a substrate as CMP-C^{14}. Very little radioactivity was found in deoxycytidine in either case. On precipitation of the extract at pH 5, two fractions were obtained both of which were necessary in succession for the overall reaction. Both were heat labile and stable to dialysis. The CMP-C^{14} could be added after the first incubation (of the pH 5 supernatant with ATP and TPN) without affecting the yield of dCMP-C^{14}. This indicates that the supernatant produces an unknown intermediate, while the pH 5 precipitate contains the actual CMP reductase. (Supported by PHS grant # C-4464.)

2 *Biochemistry*

NON-ENZYMIC INTERACTIONS OF SEROTONIN AND OTHER INDOLES WITH DPN. S.G.A. Alivisatos, G.A. Mourkides* and A. Jibril*. Mt. Sinai Med. Res. Fndn. and Hosp., the Chicago Med. School.

In the course of studies of possible enzymically catalyzed reactions of DPN with 5-OH-tryptamine (serotonin), it was observed that the latter reacts non-enzymically with DPN. When serotonin was added to a DPN solution, an intense yellow coloration appeared. Tryptamine and tryptophan reacted in a similar manner. N-methyl-nicotinamide, although less reactive, could replace DPN in these systems. Evidence accumulated so far indicates that a probable site of reaction of the indole nucleus is the pyrrolic nitrogen in its deprotonated form. The latter acts as a nucleophilic agent, attacking an electrophilic center in DPN. The participation of the nicotinamide moiety of DPN (probably carbon No. 4 of the pyridine nucleus) is indicated by the similar reaction observed with indoles and N-methyl-nicotinamide. However, in addition to changes in the visible and near U.V., the reaction mixtures exhibited also profound changes in the far U.V. region of the spectrum (at about 260 mμ). Since similar changes in the 260 mμ spectral region are also observed in AMP-indole mixtures, implication of the adenine moiety of DPN in these interactions is very likely. Attempts to isolate the products (complexes) of these interactions are now in progress. (Supported by Grants No. E-2436 from the NIH, No. G-7644 from the NSF and No. P-241 from the Am. Cancer Soc., Inc.)

3 *Biochemistry*

MECHANISM OF BACTERIOSTATIC ACTION OF PSICOFURANINE ON E. COLI. L. Slechta (intr. by M. H. Kuizenga.) Research Laboratories, The Upjohn Co., Kalamazoo, Mich.

The mechanism of antibacterial action of psicofuranine (6-amino-9-D-psicofuranosylpurine, J. Am. Chem. Soc. 81, 1767 (1959)) was studied using E. coli B. At low levels (5-10 μg/ml) psicofuranine prolonged only the lag phase of the growth curve. At higher levels (50-150 μg/ml) it also inhibited the growth rate in the logarithmic phase. The inhibition of growth by psicofuranine was completely reversed by guanine, guanosine, guanylic acid, and partially (60-80%) by deoxyguanosine and deoxyguanylic acid (at 10 μg/ml). Other purines, pyrimidines, nucleosides, nucleotides, deoxynucleosides and deoxynucleotides were ineffective. Cells grown in the presence of psicofuranine excreted xanthosine into the medium. These results suggested inhibition of the conversion of xanthylic acid to guanylic acid. Direct measurements of guanine biosynthesis from glycine-1-C^{14}, hypoxanthine-8-C^{14}, xanthine-8-C^{14} confirmed this suggestion. In all cases, decrease in guanine radioactivity and increase in xanthine radioactivity was observed in the presence of psicofuranine. Studies with tritium labeled psicofuranine showed that relatively small percentage (2-4%) of psicofuranine present in the medium was taken up by the cells.

4 *Biochemistry*

UNBALANCED GROWTH IN E. coli K-12 INDUCED BY 5-FLUOROURACIL Alexander Tomasz* and Ernest Borek. Dept. of Biochem. Columbia University, New York, N. Y.

E. coli can be protected from the bactericidal action of 5-fluorouracil (FU) by supplementing the growth medium with osmotically active solutes (Tomasz, Borek: Proc. Nat. Ac. Sci. US July, 1959). FU is active only on growing bacteria. When cells pretreated with FU are incubated in broth they spontaneously disintegrate; this growth of the cells leading to death can be prevented by supplementing the broth with osmotically active solutes or by cooling. Under these conditions all the cells regenerate the ability to form colonies within 60 minutes. In the "dying" cells the protoplasm first shrinks then bursts out into the medium. Surviving cells first elongate then split to normal sized cells. FU treated cells show high sensitivity towards UV light ($2\text{-}5 \times 10^3$ fold of normal) and towards heat and mechanical stress. FU inhibited cells accumulate nucleotide linked N-acetylhexosamine esters and peptides containing diaminopimelic, glutamic acid and alanine. The biological parameters of the accumulation together with the other features of this system are compatible with our earlier suggestion that FU induces an imbalance between the cell wall and the cytoplasmic syntheses by interfering with the function of uridine coenzymes involved in the synthesis of cell wall muco-complex.

5 *Biochemistry*

INTRACELLULAR REGULATION OF CYTIDINE NUCLEOTIDE BIOSYNTHESIS Robert B. Hurlbert, The Univ. of Texas M.D. Anderson Hosp. and Tumor Inst., Houston, Texas.

The incorporation of C^{14} from orotic acid into uridine nucleotides (U-tides), cytidine nucleotides (C-tides), and RNA uracil and cytosine was studied in Novikoff tumor cells in vitro. Unlabeled uridine in the medium caused an expected decrease in C^{14}-content of all uracil compounds and an anomalous several-fold increase in the C^{14} of all cytosine compounds, known to be derived by amination of U-tides. Unlabeled cytidine decreased greatly the C^{14} in all cytosine compounds. Chromatographic analysis of intracellular nucleotides showed that uridine and cytidine increased the amounts of U-tides and C-tides, respectively. A correlation between small increases in amount of UTP and many-fold increases in formation of CTP was noted. The maximum spec. act. of CTP was only 0.4 that of UTP. Similar results obtained with soluble enzymes showed the formation of C-tides from C^{14}-orotic acid or from C^{14}-UMP to be stimulated by U-tides and inhibited by C-tides, provided the amination enzyme was not saturated. Interpretation: The biosynthesis of C-tides via orotic acid is governed by the intracellular concentration of C-tides, which appear to be partly derived via an independent pathway. (American Cancer Soc. grants P-146, A and B)

6 *Biochemistry*

CHANGES IN THE NUCLEOTIDES OF STORED HUMAN BLOOD. Charles Bishop, David M. Rankine and John H. Talbott. (intr. by W.D. Langley), Buffalo Gen. Hosp. and Univ. of Buffalo School of Med., Buffalo, N.Y.

The nucleotide pattern of whole blood changes as soon as the blood is withdrawn and placed in storage. The present study was designed to follow these changes quantitatively. Bloods were collected in ACD solution and stored either at 4° for 8 weeks or at 37° for 3 days. In both series, column chromatography revealed that the ATP concentration fell progressively while AMP increased and IMP appeared and increased. Later on, AMP and IMP also decreased. IMP was converted to hypoxanthine almost quantitatively since the sum of ATP + ADP + AMP + IMP + Hx remained constant for some time. GTP was also broken down in the deteriorating blood system but DPN and TPN remained fairly constant. The changes at both temperatures were roughly parallel, but quantitative differences were apparent. Other experiments showed that hemolysis or the inhibition of glycolysis by iodoacetate caused a prompt loss of ATP. Methylene blue plus glucose did not alter the nucleotide pattern.

7 *Biochemistry*

STUDIES OF THYMIDYLATE KINASE ACTIVITY IN MAMMALIAN TISSUES. Howard H. Hiatt and Tadeusz B. Bojarski*. Harvard Medical School and Beth Israel Hospital, Boston, Mass.

Using the procedure of Lehman et al (JBC,233,163,1958), we have found thymidylate kinase activity of 7-13 and 1-10 units/gm protein in adult rat liver and kidney,respectively. In tissues of fetal and neonatal rats 100-400 u/gm were found. Activity in regenerating liver began to rise 18 hours after hepatectomy, reached 150-300 u/gm between 24 and 60 hours, and by 96 hours approached that of resting liver. The increase was prevented by injecting 5-fluorouracil (FU),150 mg/kg,after hepatectomy. After thymidine injections up to 100 u/gm were found in livers of FU-treated,hepatectomized rats, and in livers and kidneys of normal unoperated rats. The following suggests that the increase after thymidine is attributable at least in part to enzyme stabilization: 1) activity disappeared from tissue slices incubated in buffer for 3 hours, but not from slices incubated with thymidine; 2) a decrease of kinase specific activity on dilution of liver extract was prevented by the presence of boiled extract, thymidine, or thymidylate in the diluent; 3) thymidine or thymidylate in the homogenizing medium but not in the assay led to increased activity in normal tissues. (Aided by grants from Amer. Cancer Soc. and U.S.P.H.S.).

8 *Biochemistry*

POOL SIZES OF ACID-SOLUBLE NUCLEOTIDES OF E. COLI AS A FUNCTION OF GROWTH RATE. J.S. Franzen* and S.B. Binkley. Dept. of Biochem., Univ. of Ill., Coll. of Med., Chicago, Ill.

It is known that bacterial cell size and composition are functions of the rates of growth afforded by various media (Schaechter, et al. J. Gen. Mic. 19:592,1958). The acid soluble nucleotides of E. coli were studied in this regard. The nucleotides were resolved with Dow-1 (formate) and quantitated by UV absorbance measurements. ATP was assayed in addition by the firefly luciferase method. It was found that the cell dry weight was an exponential function of the growth rate expressed in doublings per hour. The ratio of ATP to ADP to AMP was about 100 to 12 to 2 under all conditions of growth studied. The content of ATP per cell showed about the same increment of change with growth rate as did cell mass. The UTP-UTP content increased with growth rate to a greater degree. A medium of high osmotic pressure constructs the cells and thereby concentrates the pool materials. Cells growing in the presence of 1.5% Casamino acids accumulate a nucleotide which at this time has not been identified. It is not identical with any of the nucleic acid nucleoside monophosphates, DPN, nor TPN. It is concluded that as the cells become larger they maintain the internal concentration of nucleotides.

9 *Biochemistry*

ISOLATION OF NUCLEOTIDES WITH AMINO ACID COMPONENTS FROM LIVER. David R. Wilken* and R. G. Hansen, Michigan State University, East Lansing, Michigan

Perchloric acid extracts of bovine liver were chromatographed for nucleotide components on Dowex-1 formate resin columns employing the formic acid and ammonium formate elution systems of Hurlbert et al. (J.B.C. 209, 23, 1954). After adsorbing the nucleotides on norit and eluting them with aqueous pyridine they were chromatographed on paper. During separation by three chromatographic solvent systems and also by paper electrophoresis, reaction with ninhydrin coincided with ultraviolet absorption. Bands which reacted with ninhydrin were eluted from paper chromatograms. The ultraviolet absorbing moiety was identified by absorption spectra and chromatography after acid hydrolysis and appeared to be adenine in two different compounds. In both, ribose and phosphorus were identified by conventional colorimetric methods. The amino acids liberated by acid hydrolysis have been tentatively identified by two dimensional chromatography of the free amino acids and of their DNP derivatives.

10 *Biochemistry*

ENZYMATIC SYNTHESIS OF IMIDAZOLEACETIC ACID RIBOTIDE. G. M. Crowley (intr. by G. de la Haba). Natl. Insts. of Health, Bethesda, Md.

An enzyme has been purified 60 fold from the 100,000 xg supernatant fraction of rabbit liver which catalyses the following reaction: Imidazoleacetic acid-C^{14} + PP-ribose-P $\xrightarrow{ATP}$ Imidazoleacetic acid ribotide-C^{14}. Tentative identification of the product is based on Dowex 1 acetate chromatography and paper electrophoretic mobility. Treatment of the ribotide with acid phosphatase yields a product identical to synthetic ribosyl imidazoleacetic acid (H. Bauer, Biochim. et Biophys. Acta 30, 219, 1958) in paper chromatography and electrophoresis. PP-ribose-P and ATP are required for maximum rates of ribotide synthesis both with the crude liver supernatant and the purified enzyme. Under the assay conditions used the rate of ribotide synthesis with PP-ribose-P alone is less than 5% of that when ATP is also present. Other nucleotides, versene and pyrophosphate are much less effective than ATP. P^{32} from uniformly labeled ATP^{32} is not incorporated into the product. This reaction is presumably responsible for the formation of ribosyl imidazoleacetic acid excreted by rats following administration of histamine or imidazoleacetic acid (Tabor, et al., J. Am. Chem. Soc. 77, 505, 1955). Further characterization of the enzyme and the ribotide is in progress.

11 *Biochemistry*

ON A NEW NUCLEOTIDE CONTAINING INOSITOL AND ITS ROLE IN PHOSPHATIDE BIOSYNTHESIS. Colvin M. Redman* and Lowell E. Hokin. Dept. of Physiological Chemistry, University of Wisconsin, Madison, Wisconsin.

After incubation of duck pancreas slices with inositol-2-H^3, ion exchange chromatography of the trichloroacetic acid-soluble fraction gave a sharp radioactive peak which showed U.V. absorption. The absorption spectrum of this compound was identical to that of cytidine nucleotides at pH 2 and 7. Phosphorous analysis gave 1.90 moles of phosphate per mole of cytosine, and, after hydrolysis in 6 N HCl for 48 hours at 115°, microbiological assay gave 0.94 moles of inositol per mole of cytosine. When the P^{32}-labeled compound was incubated with liver, kidney or pancreas homogenates appreciable radioactivity was incorporated into phosphatidyl choline and phosphatidyl ethanolamine and less into phosphoinositide. In a deoxycholate extract of pancreas microsomes, P^{32} from the labeled compound was incorporated primarily into phosphoinositide; this incorporation was stimulated by inositol. The results indicate that an inositol-containing nucleotide, probably cytidine diphosphate inositol, can be an intermediate in phosphatide biosynthesis. (Supported by grants from the N.I.H., Cerebral Palsy Research and Educational Foundation, and W.A.R.F. The authors are grateful to Dr. Laurens Anderson for the inositol assays.)

XUM

12 *Biochemistry*

STUDIES ON DEOXYCYTIDYLATE DEAMINASE AND THYMIDYLATE SYNTHETASE. Frank Maley* and Gladys F. Maley. Div. of Laboratories & Research, New York State Dept. of Health, Albany.

Continued studies on deoxycytidylate deaminase demonstrate that this enzyme appears at about 18 hours after partial hepatectomy and increases in specific activity until 48 hours, then decreases. Similar results were obtained with thymidylate synthetase, except that in this case the specific activity attains a maximum at about 30 hours. The appearance of the enzymes at a period when DNA synthesis is initiated suggests their participation in this process. An assay for thymidylate synthetase employing C-14 formaldehyde, tetrahydrofolate, and deoxyuridylate will be described. In studies on rat embryo supernatants, which are an excellent source of thymidylate synthetase, it was found that uridylate is methylated to form thymine ribonucleoside 5'-phosphate. The nucleoside of the above compound was identified by 2-dimensional chromatography followed by radioautography, electrophoresis in 1 per cent borate, and the isolation of sufficient compound to obtain 280/260, 250/260 ratios. Other radioactive compounds were revealed on the 2-dimensional chromatogram, two of which are possibly 5-hydroxymethyluridine and 5-hydroxymethyluracil. The methylation of deoxyuridylate was found to be similarly complex, yielding possibly hydroxymethyldeoxyuridylate and other unidentified compounds, in addition to thymidylate.

13 *Biochemistry*

PURINE NUCLEOTIDE PYROPHOSPHORYLASES OF SALMONELLA TYPHIMURIUM. G. P. Kalle, J. S. Gots, and C. Abramson.* Dept. of Microbiology, School of Medicine, Univ. of Penna., Phila., Pa.

In some cases, resistance to purine analogues results in loss of specific nucleotide pyrophosphorylases. Genetic fractionation of these enzymes was attempted by isolating mutants of Salmonella typhimurium, strain LT-2, resistant to different purine analogues. Assay of enzyme was based on the PRPP disappearance in presence of purines and on formation of nucleotides by cell-free extracts. Mutants resistant to 2,6-diaminopurine (DAP), 6-mercaptopurine (MP), and 8-azaguanine (AzG) lacked capacity to convert these analogues to their respective nucleotides. The DAP-resistant mutant showed reduced capacity to form AMP, but was able to catalyze guanine (Gu), hypoxanthine (Hx) and xanthine (Xa) to their respective nucleotides. The extracts of the MP-resistant mutant failed to catalyze the reaction of PRPP with Hx, MP, Gu, and AzG. This mutant was also cross-resistant to AzG. An AzG-resistant mutant lacked the activity for Azaguanylic, and GMP, but retained activity for formation of 6-MP-ribotide, IMP, AMP, and XMP. This mutant was sensitive to AzG. These results indicate presence of at least four different nucleotide pyrophosphorylases in S. typhimurium.

14 *Biochemistry*

Purine Nucleotide Biosynthesis in the Rabbit Reticulocyte. Jean L. Cook*, Bertram A. Lowy* and Irving M. London, Depts. of Medicine and Biochemistry, Albert Einstein College of Medicine, Bronx Municipal Hospital Center, New York.

The mature rabbit erythrocyte is incapable of de novo synthesis of the purine portion of ATP and GTP in vitro (Ann. N.Y. Acad. Sci. 75, 148 (1958)). The rabbit reticulocyte (induced by bleeding or phenylhydrazine), however, has now been shown, in vitro, to synthesize the purine portion of ATP and GTP de novo from its precursors sodium formate-C^{14}, glycine, $NaHCO_3$, L-aspartic acid and L-glutamine. A higher specific activity is found in the adenine of ATP than in the guanine of GTP. On degradation of the adenine, the radioactivity is found to be equally distributed between C_2 and C_8. In the mature rabbit erythrocyte, completion of the synthesis of the purine ring from added 4-amino-5-imidazole carboxamide (AICA), or ribosyl-AICA, and sodium formate-C^{14} occurs, and the guanine of GTP is more highly labeled than is the adenine of ATP. The reticulocyte has now also been found to utilize AICA and ribosyl-AICA, but here the adenine of ATP is found to be more highly labeled than the guanine of GTP. The presence in the erythrocyte preparations of a small admixture of leucocytes makes only a negligible contribution to the specific activity of the isolated nucleotides. (Supported by grants AEC-AT-(30-1)-1855 and USPHS A-2655.)

15 *Biochemistry*

THE CONVERSION OF THYMIDINE TO THYMIDINE NUCLEOTIDES AND DNA IN VIVO. R.L. Potter and O.F. Nygaard,* Radiation Lab. and the Dept. of Biological Chemistry, Univ. of Michigan.

Thymidine nucleotides have been isolated from the acid-soluble fraction and the DNA of rat spleen and thymus at various times following the injection of C^{14}-thymidine in an attempt to establish whether the specific activity of the nucleotide is high enough to account for the incorporation of radioactivity into DNA. The specific activity of the thymidylic acid in the newly synthesized DNA can be calculated from the fractional turnover of labeled DNA (Nygaard and Potter, Radiation Research, in press). In spleen the soluble thymidine nucleotides rapidly reach the same specific activity as thymidine. The specific activity of the thymidine triphosphate is equal to the specific activity of the thymidylic acid of the newly synthesized DNA, showing that the soluble nucleotide can be considered to be the precursor. In thymus the thymidine nucleotides do not reach equal specific activity for at least an hour and are still below the specific activity of the thymidine. The specific activity of the thymidine triphosphate is an order of magnitude too low to account for the radioactivity of the newly synthesized DNA. It is concluded that spleen has only a dynamic pool of thymidine nucleotides while thymus has in addition a relatively large static pool of these compounds.

16 *Biochemistry*

METABOLIC ALTERATIONS ACCOMPANYING FATTY INFILTRATION OF LIVER INDUCED BY OROTIC ACID. R. E. Handschumacher, W.A. Creasey and L. Hankin (intr. by A. D. Welch). Dept. of Pharmacology, Yale Univ. School of Medicine and Conn. Agricultural Exp. Station, New Haven, Conn.

The rapid and severe fatty infiltration of the livers of rats fed a purified diet containing 1% orotic acid affords a valuable tool for studies on metabolic imbalance in nucleotide and fat metabolism. A report on the effects of adenine and other dietary supplements on fat content and certain aspects of pyrimidine metabolism has been given (Handschumacher et al., Proc. Nat. Acad. Sc., Feb., 1960). Additional studies indicate that simultaneous ingestion of the orotic acid and the purified diet is essential for accumulation of fat; coprophagy nullifies the effects of orotic acid on fat deposition. Analysis of livers from rats receiving the orotic diet indicate a reduction in niacin, iron and phospholipid content. Furthermore, the incorporation in vivo of acetate-2-C^{14} into phospholipids is decreased, while that into neutral fat is increased. The characterization of the fatty acids from various lipid fractions by gas chromatography will be described. Particle-free preparations from the livers of orotic-fed animals have one-third the capacity to convert uridylic acid to cytosine derivatives, which may reflect a depressed synthesis of cytidine-coenzymes essential for phospholipid biosynthesis.

17 *Biochemistry*

SYNTHESIS AND ENZYMIC INHIBITIONS OF RIBONUCLEOTIDE DERIVATIVES OF PURINE ANALOGS. Alexander Hampton* (intr. by G. B. Brown). Sloan-Kettering Inst. for Cancer Research, N.Y.21,N.Y.

Growth inhibition by purines can involve biosynthesis by the susceptible organism of 5'-phosphoribosyl derivatives. To assist study of their biological properties, methods for the synthesis of such nucleotides have been developed, with primary emphasis on nucleotides derived from purines active against human leukemia. Treatment of 6-chloro-9-β-D-ribofuranosylpurine with p-toluenesulfonic acid in acetone gave the 2',3'-O-isopropylidene derivative in 80% yield; phosphorylation of this with tetra-p-nitrophenyl pyrophosphate and removal of blocking groups gave 20% of purified 6-chloro-9-β-D-ribofuranosylpurine 5'-phosphate. 6-Mercapto-, and 2-amino-6-mercapto-9-β-D-ribofuranosylpurines were converted in good yield to the respective 2',3'-O-isopropylidene derivatives by a modification of the p-toluenesulfonic acid procedure, and phosphorylated as above to give "thioinosinic" and "thioguanylic" acids in 25% yields. The rate of conversion of IMP to adenylosuccinate by adenylosuccinate synthase from E. coli B was reduced by 50% with 1 equiv. of "thioinosinic acid", and that of adenylosuccinate to AMP by adenylosuccinase by 50% with 4 equiv. of "thioinosinic acid". This sequential inhibition of the two steps, from IMP to AMP, could be involved in growth inhibition by 6-mercaptopurine.

18 *Pharmacology*

NUCLEOSIDE METABOLISM BY BRAIN EXTRACTS FROM RAT AND GUINEA PIG. D. A. Rappoport, R. Fritz and A. S. Moracewski (intr. by R. A. Huggins.) Departments of Biochemistry and Psychiatry, Baylor Univ. College of Medicine, Houston, Texas.

Soluble extracts from rat and guinea pig brain metabolized purine ribonucleosides in presence of inorganic phosphate to form phosphate esters and lactic acid. Separation of these esters by paper chromatography indicated the presence of pentose phosphate, hexose phosphates and ph-glyceric acids. Deoxyribonucleosides were metabolized to a small extent by guinea pig brain extracts only. Ribose-5-phosphate was rapidly metabolized by these extracts. These studies showed that the pentose moiety of the nucleosides is transformed into a variety of phosphate esters through the action of transketolase and the glycolytic enzymes. The details of the reactions concerned with nucleoside metabolism by brain extracts will be discussed.

19 *Pharmacology*

A SENSITIVE COLORIMETRIC ASSAY FOR PYRIDINE COMPOUNDS. L. D. Saslaw* and V. S. Waravdekar. Armed Forces Institute of Pathology, Washington, D.C.

Some pyridine compounds yield conjugated aldehydes upon reaction with cyanogen bromide. It has been reported that conjugated aldehydes react with thiobarbituric acid to form intensely colored derivatives (Waravdekar and Saslaw, J. Biol. Chem. 234:1945, 1959). On the basis of these reactions, a method for estimation of nicotinamide was devised. To 0.5 ml. of an aqueous solution containing 0.0025 to 0.04 micromole of nicotinamide, 0.5 ml. of 5% aqueous cyanogen bromide was added. After standing for 20 minutes at 25°C, the mixture was vigorously aerated for 2 minutes to remove excess cyanogen bromide. The solution was mixed with 2.0 ml. of 0.6% thiobarbituric acid (pH 2.0) and was heated for 15 minutes in a boiling-water bath. On cooling, the absorbancy of the pink solution was measured at 533 millimicrons ($E_{max} = 5 \times 10^4$). This procedure was found applicable for the estimation of some pyridine nucleotides when an elevated temperature and KCN were used during cyanogen bromide addition.

201. NUCLEIC ACIDS–Purines, Pyrimidines

1 *Physiology*

NUTRITIONAL AND ENDOCRINE INFLUENCES ON URACIL INCORPORATION IN LIVER RNA. A. Cantarow, K.E.Paschkis, and T.L.Williams*. Jefferson Medical College, Philadelphia, Pa.

Active utilization of uracil for RNA synthesis in the rat appears to be a property of rapidly growing tissues. It had been shown previously that this pathway of RNA biosynthesis can be influenced by thyroidectomy, growth hormone, and testosterone. The influence of starvation, cortisone, and hypophysectomy was investigated. Starvation (4 days) was followed by increased incorporation of uracil in liver RNA of adult rats to levels approximating those observed in hepatoma and regenerating liver. Administration of cortisone (5 mg. daily, 4 days) resulted in decreased incorporation in preneoplastic (AAF-treated) but not in regenerating liver. Hypophysectomized rats showed increased incorporation in liver RNA, which decreased after administration of growth hormone. Starvation resulted in increased incorporation in transplanted hepatomas, but not in the livers of rats bearing this tumor. It would appear that a common denominator of stimulation of growth, or of increased rate of turnover of RNA, is not applicable to the phenomenon of active incorporation of uracil into RNA. (Supported by USPHS Grant C-1307).

2 *Biochemistry*

ISOLATION OF TRANSFORMYLASE AND PTEROICASES FROM SULFATHIAZOLE SENSITIVE AND SULFATHIAZOLE-RESISTANT B STRAINS OF E. COLI. T. Yokota and M.G.Sevag* Univ. Penna. School of Med. Phila.

Cell-free extracts from the sulfathiazole (ST)-sensitive B strain of E. coli saturated 20-40% with ammonium sulfate yield transformylase and only pteroicase A. Cell-free extracts from the ST-resistant strain, on the other hand, yield transformylase and pteroicase A at 20-40% saturation and pteroicase B at 60-100% saturation with ammonium sulfate. These crude enzyme fractions were further fractionated chromatographically using activated alumina and Dowex 50 columns. Purified transformylase has also inosinicase activity and contains one type of bound formyltetrahydrofolic acid. CF-cofactor content of transformylase from the ST-resistant strain is greater than that from the sensitive strain. The activity of pteroicase A is inhibited from 50-55% by 10^{-2} M of ST in the presence of 10^{-6} M of pteridine and 10^{-4} M of PAB. Pteroicase B is not inhibited under these conditions. Of the two pteridines, 2-amino-4-hydroxy-6-carboxypteridine and 2-amino-4-hydroxy-6-hydroxymethylpteridine, the former has a greater activity as substrate than the latter. 2-Amino-4-hydroxy-6-methylpteridine is inactive as substrate. (Supported by a grant from ONR.)

3 *Biochemistry*

THE METABOLISM OF 5-RIBOSYLURACIL. Adam W. Lis* and Frank Worthington Allen. Department of Biochemistry, Univ. of California School of Med., San Francisco.

A dialyzed homogenate has been prepared from a microorganism that has been isolated from creek-mud and shown to contain enzymes which catalyze the following reactions: 5-ribosyluracil ⟶ uridine ⟶ uracil. Treatment of the homogenate with ammonium sulfate yields a fraction between 40% to 60% saturation in which only the enzymatic transformation of 5-ribosyluracil ⟶ uridine is obtained. Thus, the catabolism of 5-ribosyluracil is in contrast to that of other known nucleosides in that it requires at least one intermediate reaction involving the formation of uridine before attack by nucleoside phosphorylase. The transformation requires phosphate and has a broad band type of optimum at pH 6.8 ± 0.5. In contrast to known phosphorylase reactions the activity is inhibited by arsenate. Since the ribose of 5-ribosyluracil has been allocated to position 5 by analogy rather than unequivocal synthesis it is of interest that the enzymatic transformation is strongly inhibited by 5-methyluracil (thymine) and by 5-hydroxymethyluracil. Other 4- or 5- substituted pyrimidines such as 4-methyluracil or 5-bromouracil do not significantly inhibit the transformation.

4 *Biochemistry*

THE OXIDATION OF DPNH BY HYPOXANTHINE DEHYDROGENASE FROM AVIAN KIDNEY. Erwin J. Landon (intr. by Stanley Cohen.) Dept. of Pharmacology, Vanderbilt Univ. School of Med., Nashville, Tenn.

The purified metalloflavoprotein enzyme catalyzes the conversion of hypoxanthine to uric acid with DPN as coenzyme. DPNH oxidation is catalyzed by the enzyme in the presence of the following three artificial acceptors: hydroxylamine, menadione and methylene blue. Inhibition of enzymatic activity by p-chloromercuribenzoate and CN is similar in both purine and DPNH oxidation. K_m values determined for menadione and hydroxylamine as acceptors in the DPNH oxidation reaction are identical with the values determined for them in the hypoxanthine-acceptor reaction. In the absence of an acceptor the enzyme quenches the 460 mμ fluorescence peak of DPNH. This DPNH quenching is not affected by PCMB but is inhibited by DPN. The K_m for DPNH in this reaction agrees with that determined for DPNH in the oxidation reaction. The values found appear to indicate that DPNH is much more tightly bound to the enzyme than either hypoxanthine or DPN which are the physiological substrates. DPNH has been found to inhibit the formation of uric acid by competing with DPN. Evidence is presented for a functional relation between the enzyme and lactic dehydrogenase both in vivo and in vitro in maintaining oxidized DPN for the formation of uric acid.

5 *Biochemistry*

THE ENZYMIC CONVERSION OF 6,7-DIMETHYL-8-RIBITYL-2,4-DIOXYPTERIDINE (6,7-D) TO RIBOFLAVIN. G.W.E. Plaut, Lab. Study Hered. Metabol. Disor., U. Utah Col. Med., Salt Lake City, Utah.

The conversion of 6,7-D to B_2 by sonates of A. gossypii was reported previously (JACS 81, 2025, 1959). This enzymic activity has been partially purified from A. gossypii. Studies with intact cells indicated that ring A of B_2 probably originated from four 2-carbon units (Plaut, JBC 211, 1954). Since 6,7-D already contains four of the eight carbons required to form this ring, the origin of the remaining four carbons was investigated, by study of the requirements of the conversion reaction. However, only, 6,7-D, enzyme, and buffer were needed for maximal B_2 production. Addition of coenzymes or acetyl group precursors did not enhance conversion. These results suggested that the four carbons originate from 6,7-D itself. Further evidence has been obtained with crude and purified enzyme preparations: a) 3 moles of 6,7-D are consumed per mole of B_2 formed. In addition to B_2, at least one other substance is produced; one of these is tentatively identified as 6-methyl-7-hydroxy-8-ribityl-2,4-dioxypteridine. b) B_2 formed from 6,7-dimethyl-C^{14}-8-ribityl-2,4-dioxypteridine has about twice the molar specific radioactivity of the precursor; the specific activity of the methyl groups and adjacent C 6+7 portion of ring A of B_2 is about equal to that of the labelled precursor.

6 *Biochemistry*

THE LOCALIZATION OF TRITIUM IN THE METHYL GROUP OF THYMINE. Morris Friedkin. Dept. of Pharmacology, Tufts University School of Medicine, Boston, Massachusetts.

It was of interest to determine the position of tritium in thymine derived from two sources: (1) thymine-H^3 of commercially available thymidine-H^3 prepared by catalytic exchange of tritium from the carboxyl group of acetic acid by the method of W.L.Hughes and (2) thymine-H^3 of thymidylic acid formed by an enzymatic reaction between deoxyuridylic acid, formaldehyde, and tritiated tetrahydrofolic acid (Federation Proc.,18: 230,1959).The usual chemical procedures for determining the amount of carbon-14 in the methyl group of thymine cannot be used for tritium because of tautomeric exchanges with hydrogen ions of the medium.Thymine-H^3 was mixed with thymine-2-C^{14} and then converted to 5-methylbarbituric acid by the action of "thymine oxidase"(Hayaishi and Kornberg,J.Biol.Chem.197:717, 1952). Any tritium attached to carbon at position 6 of the pyrimidine would be lost upon oxidation of thymine to 5-methylbarbituric acid.Since the ratio of tritium to carbon-14 in the barbiturate was found to coincide with the ratio of isotopes in the doubly-labeled thymine, it was concluded that all of the tritium was localized in the methyl group.This finding applied to thymine-H^3 from both sources mentioned above.(This research was supported in part by grants from USPH and NSF.)

7 *Biochemistry*

THE EFFECT OF PYRAZOLOADENINE ON CANDIDA UTILIS AND ESCHERICHIA COLI. A. S. Brecher, B. S. Baker, and M. B. Boncer (intr. by E. M. K. Geiling.) Division of Pharmacology, Food and Drug Administration, U. S. Dept. of Health, Education, and Welfare, Washington, D. C.

The intraperitoneal injection of pyrazoloadenine into rats. (Feigelson and Davidson, Cancer Res., 18:226, 1958) led to the urinary excretion of pyrazolohypoxanthine, pyrazoloxanthine, and pyrazoloisoguanine. Incubation of pyrazoloadenine with C. utilis and E. Coli for six and one days respectively have been carried out. Supernatant broths were studied in the Perkin-Elmer spectracord. No noticeable shift in the peaks were observed in comparison of curves obtained before and after incubation. Adenine, which overcomes inhibition of growth in C. utilis caused by the antimetabolite, is converted to hypoxanthine in both microorganisms. Mixtures of adenine and pyrazoloadenine exhibited a shift in the peak which has been attributed to the formation of hypoxanthine. While supernatant broths containing other compounds such as pyridoxine, thiamine, hypoxanthine, and adenosine, which also overcome the inhibitory effect of pyrazoloadenine in C. utilis were observed, there was no detectable shift in the peak.

8 *Biochemistry*

THE MECHANISM OF PEROXIDATION OF URIC ACIDS BY HEMATIN PROTEINS. R.R. Howell* and J.B. Wyngaarden. Duke Univ. Medical Center, Durham, N. C.

Uric acid oxidation occurs in the presence of H_2O_2 and various peroxidases. We have reinvestigated this reaction, and have found that it is catalyzed by hematin in the absence of protein. Hematin, derived from methemoglobin, horse-radish peroxidase or catalase was an active catalyst of uricolysis (followed as loss of absorbance at 292 mu) above pH 6-7, whereas heme-proteins had pH optima of 5-5.5. All 8-oxypurines were oxidized, unless both N-7 and N-9 were blocked, as in tetramethyluric acid. The reaction sequence involved initial formation of a hematin-H_2O_2 intermediate which then oxidized uric acid. It is proposed that the addition product, probably $\equiv Fe^{3+}$. OOH, generates an hydroxyl free radical which then attacks purine C-4 (or C-5) because of the carbanion character of this carbon in the canonical form in which the N-7 (or N-9) proton is labelized by the 8-carbonyl. This mechanism is supported by the observation that uric acid is oxidized at pH 5 in H_2O_2 only after exposure of H_2O_2 to UV light (known to generate OH^o) and that purines lacking a free N-7 or N-9 position or an 8-oxy group are more resistant to oxidation than uric acid. (Supported by USPHS Grant No. A-1391).

9 *Biochemistry*

A MECHANISM OF RESISTANCE TO FLUORINATED PYRIMIDINES. K.L. Mukherjee*, K-U. Hartmann*, George Kaldor*, and Charles Heidelberger. McArdle Lab. Univ. of Wisconsin, Madison.

Reichard et al (Nature 183, 939 (1959) have reported that tumors resistant to 5-fluorouracil (FU) are incapable of converting it into its ribonucleotide. We have developed a line of the Ehrlich ascites carcinoma that is resistant to fluoropyrimidines by a different mechanism. This tumor utilizes uracil and FU for RNA biosynthesis to only a slightly lesser extent than does the susceptible tumor. At chemotherapeutic doses FU does not affect RNA biosynthesis in either tumor. However, the incorporation of formate into DNA thymine is inhibited in the susceptible but not in the resistant tumor. It is known from earlier work that 5-fluorouridine-5'-monophosphate (FUDRP) is a potent inhibitor of thymidylate synthetase. Nevertheless, this nucleotide is produced in equal amounts from FU by both tumors. Thymidylate synthetase activity was measured in 100,000 x g supernatant fractions from sonically disrupted susceptible and resistant tumor cells. In the enzyme from the susceptible tumor FUDRP (prepared synthetically in this laboratory) inhibited to a 1000-fold greater extent than it did in the resistant tumor. Thus the resistance to fluoropyrimidines in this tumor line results from an alteration in the active site of thymidylate synthetase such that the enzyme can distinguish between UDRP (the natural substrate) and FUDRP.

10 *Biochemistry*

ALTERNATIVE PATHWAYS FOR BIOSYNTHESIS OF THYMIDYLIC ACID. Van R. Potter, H. C. Pitot*, A. B. McElya*, and P. A. Morse*. McArdle Memorial Lab., U.Wis. Med.School, Madison, Wisconsin

Thymidylic acid can be formed from thymidine or from deoxyuridylic acid, while the latter can be formed from uridylic acid or deoxycytidylic acid. Thymidine is either not available to most tissues or is available at very low levels because of the unique capacity of the liver to clear the blood of this compound and convert it to CO_2. It appears that various tissues in the body differ greatly in the ratios between the rates of the different reactions leading to thymidylic acid. These ratios have been studied in vitro by assays for individual enzymes, and in vivo by injecting 10 microcuries H^3-deoxycytidylic acid into rats and measuring the specific activity of the thymine and cytosine of DNA isolated at various times after injection. Remarkably little deamination of deoxycytidine occurs and the compound is apparently not catabolized in other ways. Since in the young adult rat only the thymus and bone marrow contain deoxycytidylic deaminase, it might be expected that in all other tissues the ratio of the specific activities of DNA thymine to DNA cytosine would be zero in the whole animal experiments, but spleen, which lacks the enzyme, showed a ratio nearly equal to that of thymus at 24 hours. It seems possible that deoxyuridine may pass from one tissue or cell type to another to account for the results.

11 *Biochemistry*

A MECHANISM OF RESISTANCE TO 6-THIOGUANINE. R.W. Brockman and Patricia Stutts (intr. by H.E. Skipper), Southern Research Institute, Birmingham, Ala.

Soluble enzyme preparations (designated E) from neoplastic cells resistant to 6-mercaptopurine (MP) and 8-azaguanine (AZAG) were less active in catalyzing formation of 5'-ribonucleotides than were E from drug-sensitive neoplasms (Can. Res. 19, 856, 1959). 6-Thioguanine-resistant Ehrlich ascites cells formed less 6-thioguanylic acid (TGMP) in vivo and incorporated less 6-thioguanine (TG) into nucleic acids than did sensitive cells (Sartorelli et al., Can. Res. 18, 1232, 1958). E from L1210 leukemic cells resistant to TG, MP and AZAG (lines of L. W. Law) had markedly less capacity to catalyze formation of IMP, GMP and TGMP from corresponding bases than did E from drug-sensitive L1210 cells. Adenine was converted to AMP equally well by E from sensitive and resistant cells. E from sensitive and resistant cells exhibited little capacity to degrade natural purines and TG under the experimental conditions used. E from liver and spleen catalyzed ribonucleotide formation but also had significant catabolic activity. Resistance to TG in L1210, like resistance to MP and AZAG, is thus seen to be accompanied by decreased ribonucleotide pyrophosphorylase activity.

12 *Biochemistry*

STUDIES ON THE FATE OF TRITIATED THYMIDINE IN PARAMECIUM AURELIA. J. Berech, Jr.* and W. J. van Wagtendonk. Dept. of Zool., Indiana University, Bloomington, Indiana.

Tritiated thymidine has been used to study the nuclear reorganization process, autogamy, in Paramecium aurelia. Paramecia were grown axenically in a medium containing 10-50 μc/ml of the tracer for 5 days. After removal of external tracer, the cells were transferred to either (1) fresh medium, (2) exhausted medium, or (3) isotonic salt solution (each medium without tracer), and incubated for appropriate time intervals to allow autogamy to occur. In no case was there a preferential re-utilization of the label for the formation of the new macronucleus. Radioautographs of Feulgen-stained cells showed significant labeling of the cytoplasm. The possibility that this label is associated with DNA or RNA is under investigation. The nucleic acid of radioactive Paramecia has been fractionated and three fractions, TCA-soluble, DNA-bases, and RNA-tides have been separated by one dimensional paper chromatography. Scanning of the chromatograms shows that UMP, derived from RNA is labeled. Cytosine derived from DNA is not labeled. It was uncertain whether CMP from RNA was labeled. These results indicate that thymidine is converted to a uridine derivative which is incorporated into RNA. (Supported by grants from the Nat. Inst. of Health and the Nat. Science Found.)

13 *Biochemistry*

EFFECT OF URETHANE UPON PYRIMIDINE BIOSYNTHESIS. Edward Bresnick* (intr. by Gertrude B. Elion). Wellcome Research Labs., Tuckahoe, N.Y.

A growing body of evidence has indicated that urethane is involved with pyrimidine metabolism. The possible structural analogy between urethane and carbamyl phosphate suggested that it might be acting as an antagonist of this important pyrimidine precursor. Ehrlich ascites cells by treatment with sonic oscillation and centrifugation (600 x g) yielded a supernatant fraction with ureidosuccinate synthetase activity. Two methods were employed in the determination of ureidosuccinate formation from carbamyl phosphate (20 uM) and l-aspartic acid (30 uM) at pH 8.8 in Tris buffer and isolation of ureidosuccinate-C^{14} produced from dl-aspartate-4-C^{14}. A stimulation of ureidosuccinate formation was observed when urethane, at an inhibitor/substrate ratio of 50/1 or higher, was added to the incubation mixture containing subsaturation levels of carbamyl phosphate (below 20 uM). This stimulation could be partially correlated with a protection by urethane of carbamyl phosphate, at subsaturation levels, from decomposition by phosphatases present in the enzyme source. At and above saturation levels of carbamyl phosphate urethane at inhibitor/substrate ratios of 40/1 or higher inhibited ureidosuccinate synthetase. Urethan may therefore be considered an antagonist of this enzymatic reaction.

14 *Biochemistry*

ENZYMATIC SYNTHESIS AND DEMETHYLATION OF 6-METHYLAMINOPURINE. Charles N. Remy. V.A. Hosp. and State Univ. of N.Y., College of Med., Syracuse, N.Y.

Resting cell suspensions of E. coli strains B, 15T⁻, and B-96 in Tris buffer demethylate 6-methylaminopurine (MeAd) but not 6-dimethylaminopurine (DiMeAd) or 2-amino-6-methylaminopurine (MeDAP) to yield hypoxanthine. The ability of various cell preparations to demethylate MeAd parallels their capacity to deaminate adenine. The rate of demethylation is increased by glucose and phosphate but is unaffected by a N_2 atmosphere, cyanide, or A-methopterin. Demethylation is inhibited by DiMeAd, MeDAP, and adenine; in turn, the methylpurines inhibit the deamination of adenine. The methyl group is not released as free HCHO. Synthesis of MeAd-CH_3-C^{14} was demonstrated by culturing E. coli 15T⁻ in a thymine deficient medium supplemented with adenine and methionine-CH_3-C^{14}. Since adenine inhibited de novo purine synthesis, formation of MeAd nucleotides, and demethylation of free MeAd, MeAd-CH_3-C^{14} was present in the final growth medium in far greater quantities than in the DNA of the cells. (Supported by U.S.P.H.S. grant RG-6528)

15 *Biochemistry*

THE ROLE OF BIOTIN IN THE UTILIZATION OF AMINOIMIDAZOLE FOR PURINE BIOSYNTHESIS. Albert G. Moat and Floyd Nasuti*. Dept. Microbiology, Hahnemann Med. Coll., Philadelphia, Pa.

Previous reports from our laboratory indicated a role for biotin in the synthesis of purines by Saccharomyces cerevisiae as evidenced by the accumulation of aminoimidazole (AI) or its riboside under the conditions of biotin deficiency. To obtain additional evidence for the role of biotin in the utilization of AI for purine synthesis, a system was studied in which the conversion of AI to purines was stopped at aminoimidazole-carboxamide (AICA) by means of amethopterin inhibition. AI and AICA were distinguished by means of their ultraviolet absorption spectra and their spectra in the visible range after diazotization and coupling with the Bratton-Marshall reagents. In the presence of glucose, phosphate buffer, and ammonium sulfate, biotin-deficient cells accumulate AI. In the presence of added biotin, the accumulated AI is utilized and, in the presence of amethopterin, accumulates as AICA. Free aminoimidazole added as substrate is also utilized for AICA synthesis in the presence, but not in the absence of biotin. Nitroimidazole is converted to AI by this organism. Bicarbonate and aspartate, known to be involved in the conversion of AI to AICA, exert little or no effect upon the conversion of AI to AICA in the absence of biotin. However, in the presence of biotin the rate of conversion is enhanced.

16 *Biochemistry*

REVERSING AGENTS FOR ALCOHOL TOXICITY. Edwin M. Lansford Jr.† Ira D. Hill† and William Shive. Clayton Foundation Biochemical Institute and Dept. of Chemistry, U. of Texas, Austin.

Glutamine was identified as a reversing agent for inhibitory metabolic effects of alcohol in a search for possible therapeutic agents for alcoholism. Further study shows that liver and other natural extracts reverse ethanol (5.0% v/v) inhibition of growth of L. arabinosus 17-5 in an amino acid medium containing glutamine, vitamins, purines and uracil. The active principles in liver can be replaced by a riboside (e.g. adenosine, guanosine, inosine, cytidine, or uridine) but not by a ribotide, deoxyriboside, or deoxyribotide. The phosphates were previously shown not to be utilized by the organism. The liver activity is attributable to various ribosides as shown by paper chromatography. Without the purine-pyrimidine supplement, a combination of a purine riboside with a free pyrimidine or a pyrimidine riboside with a free purine is required for reversal of growth inhibition by alcohol. These results suggest that alcohol inhibits purine and pyrimidine synthesis at the stage of incorporation of 5-phosphoribosyl units but does not prevent transribosidation. Enzyme studies are in progress to locate the metabolic site of alcohol inhibition, and further study of purine and pyrimidine riboside and ribotide derivatives alone and to augment glutamine therapy in conditions amenable to glutamine therapy is suggested.

XUM

17 *Biochemistry*

INHIBITION OF XANTHINE OXIDASE BY KINETIN AND KINETIN ANALOGUES Thomas R. Henderson (intr. by J. W. Foster.) Clayton Foundation Biochem. Institute, Dept. of Chemistry, University of Texas, Austin.

A variety of 6-(substituted)purines have been shown to equal kinetin in biological activity, or to have significant activity in somewhat higher concentrations in several kinetin test systems. In the alkyl substituted series, the 6-monoalkylaminopurines appear to be considerably more active than the dialkylamino- and alkylthiopurines, and the optimum alkyl group for biological activity is C_5 to C_6. It appears that a significant correlation can be drawn between the "activity spectrum" of 6-(substituted)purines, and their inhibitory properties on milk xanthine oxidase. The monoalkylaminopurines are oxidized by this enzyme, and the oxidation products appear to be more potent inhibitors of this enzyme than the parent purines. Alkylthiopurines and dialkylaminopurines inhibit the enzyme to a similar extent, but they are not oxidized under these conditions, apparently due to a lack of tautomeric shifts which are necessary for oxidation. These results suggest that the mechanism of action of kinetin and related compounds in some biological systems may be through inhibition of xanthine oxidase or closely related flavoproteins. In this manner, these 6-(substituted)purines may affect cell division and differentiation.

18 *Biochemistry*

SPECIFICITY OF THE PYRIMIDINE CATABOLIZING SYSTEM OF RAT LIVER. Harold W. Barrett, S. N. Munavalli* and Philip Newmark. Department of Biochemistry, University of Kansas, Lawrence.

Barrett and West have suggested that catabolism of some 5-halogenopyrimidines *in vivo* in the rat proceeds via reduction to the 5-halogeno-5,6-dihydropyrimidines followed by dehydrohalogenation to uracil, then via the reductive pathway for thymine and uracil degradation which was elucidated earlier by the Finks and their co-workers. In order to learn more about the catabolism of unnatural pyrimidines in rat liver, we examined the effects *in vitro* of various natural and unnatural pyrimidines on the degradation of uracil-2-C^{14} and thymine-2-C^{14}, as measured by the release by $C^{14}O_2$. It seemed reasonable that breakdown of the natural pyrimidine substrates might be inhibited by those unnatural pyrimidines which the liver enzyme system could also degrade. With a high speed centrifugal supernatant fraction of liver homogenate as the enzyme system, all 5-monosubstituted uracils inhibited both uracil and thymine degradation to varying extents. Uracil and thymine inhibited the degradation of each other reciprocally, but not equally. Cytosine and its derivatives, and analogs of uracil which were substituted in positions on the pyrimidine ring other than 1 or 5 showed little or no inhibitory activity. Cytosine-2-C^{14} was not degraded by the enzyme system.

19 *Biochemistry*

SOURCES OF THE 5-METHYL CARBON OF DESOXYRIBONUCLEIC ACID THYMINE IN BONE MARROW. I. Korc,* M. Muniz,* B. Mendioroz,* and J. R. Totter. University of Montevideo, Uruguay.

Tryptophan was shown by dilution of radioactivity to be less than 1% as effective as formate when incubated with rabbit bone marrow and C^{14} radioformate *in vitro*. However, when the marrow was obtained from an animal injected with 1.5gm. of tryptophan 5hrs. previously, similar experiments gave an efficiency of 6% for the amino acid. Tryptophan pyrrolase activity in bone marrow of untreated or tryptophan injected animals could not be demonstrated and must be very low. Histidine was inactive as a diluent for radioformate unless previously incubated with liver to produce formamidino glutaric acid. The latter compound was more effective as a "diluent" at high than at low concentrations in contrast to inactive formate which gave consistent dilutions at all concentrations tested. The results indicate that formate does not saturate the tetrahydrofolic acid system in bone marrow even though it is an excellent source for the 5-methyl group of thymine. Formamidino glutaric acid may be an important if not a major source for the 5-methyl group. The pool size of formate was indicated to be about 0.03 μ moles and the thymidine pool about 0.02μ moles per μ mole of DNA phosphorus in rabbit bone marrow. (Financial assistance was given by the Rockefeller Foundation and by the U.S. Atomic Energy Commission.)

20 *Biochemistry*

INTRODUCTION OF 5-IODOURACIL INTO DNA OF MAMMALIAN CELLS. E.Gambetta Hampton, Marvin A.Rich and Maxwell L.Eidinoff (intr. by Ralph K.Barclay), Sloan-Kettering Institute for Cancer Research, N.Y.

A sensitive method requiring approx. 10^6 cells was developed and tested for measuring the incorporation of unnatural base into DNA (molar ratio of 5-iodouracil to 5-iodouracil plus thymine). This ratio is calculated from the specific activity (cpm/γ) of cellular DNA obtained as DNA-salmine precipitate by a modification of the McIntire-Sproull procedure (Proc.Soc.Exp.Biol.and Med., 95, 458, 1957). H.Ep.#1 cells were incubated in Eagle's medium (plus 10% horse serum) containing 5-iododeoxyuridine labeled with I131 . Incorporation of the 5-iodouracil moiety into the DNA of H.Ep.#1 cells reached a plateau at approx. 4, 10 and 40% after 48 hours incubation in medium containing 1, 5 and 50 γ/ml respectively. At conc. of IUDR in the range 5-50 γ/ml, addition of equimolar thymidine conc. reduced the incorporation of unnatural base by approx. 60%. Increasing the specific activity of IUDR from 0.10 to 10 μc/mg did not alter the incorporation of the iodouracil moiety.

21 *Pharmacology*

ADENINE TOXICITY IN MAMMALIAN CELLS CULTURED IN VITRO. Lewis Aronow (intr. by Avram Goldstein.) Dept. of Pharmacology, Stanford Univ., Calif.

Adenine, at a concentration of 10^{-3} M, has been reported to be toxic to mammalian cells cultured in Eagle's medium despite the fact that this compound can be utilized by the cells. In an effort to determine the mechanism of this toxic effect, a series of compounds were tested for their ability to support the growth of mouse fibroblasts ("L" cells) in the presence of 10^{-3} M adenine. No purine derivative (ribosides or ribotides) had any effect, nor did any purine precursor. Glycine and serine were likewise without effect, but the toxic effect was reversed by uridine or cytidine at 10^{-4} M. Uracil or cytosine were without effect, even at 10^{-3} M. It is suggested that high levels of adenine deplete the cells of their pool of available phosphoribosylpyrophosphate. Supplying pyrimidines in the form of ribosides would then allow the cells to continue nucleic acid synthesis even in the absence of PRPP. Nucleic acid purines would derive from the adenine itself. The system is in some respects similar to that described by Gots and Goldstein (Science 130: 622, 1959) for certain mutant strains of E. coli. (Supported by USPHS Grant CY2797.)

22 *Pharmacology*

DIRECT SYNTHESIS OF PURINES FROM AMINO ACIDS. J.Oró and A.P. Kimball (intr. by Richard A. Seibert). Dept of Chemistry, Univ. of Houston, Houston, Texas.

Amino acids can be synthesized from C_1 and N_1 compounds under a variety of possible primitive earth conditions (S. Miller, J. Am. Chem. Soc., 77:2351, 1955; J. Oró et al., Arch. Biochem. Biophys., 85: 115, 1959). Purines have now been synthesized by heating at 100°C and 220°C mixtures containing amino acids in either aqueous or anhydrous systems. Adenine and guanine have been found among the products of the reaction. These two purines have been tentatively identified by the ultraviolet absorption spectra in hydrochloric acid and by paper chromatography. Each of the following mixtures produced at least one purine or aminopyrimidine as detected by paper chromatography and reaction with the silver nitrate-bromo phenol blue reagent: Urea-glycine-formamide (220°C), urea-glycine-ammonium formate (220°C), urea-glycine-alanine (220°C), urea-glycine-β-alanine (220°C), urea-serine (220°C), ammonium carbonate-serine-formamide-water (100°C).

202. NUCLEIC ACIDS–RNA

Physiology

CHARACTERIZATION OF RIBONUCLEIC ACID (RNA) SYNTHESIZED IN PHAGE INFECTED E. COLI. M. Nomura*, B. D. Hall, and S. Spiegelman. Dept. of Microbiology and Dept. of Chemistry, University of Illinois, Urbana, Illinois.

The RNA formed in E. coli after infection with phage T2 and identified by P^{32} labeling (E. Volkin and L. Astrachan, Virology, 2, 149, 1956) has been further investigated. It was found to occur mainly in the ribosome fraction, provided the disruption and fractionation of the cells are carried out in a medium containing a high concentration of Mg^{++} (10^{-2}M $MgCl_2$ in 10^{-2}M tris buffer at pH 7.3). The newly synthesized (P^{32} labeled) RNA in ribosome fraction shows the electrophoretic behavior of a nucleoprotein. This RNA was isolated as free RNA by treatment of the ribosome fraction with aqueous phenol and characterized with respect to electrophoretic mobility, sedimentation velocity, and apparent base composition. In each of these properties the P^{32} labeled RNA differs markedly from the pre-existing RNA.

Thus, its electrophoretic mobility is higher and its average sedimentation constant is lower than those of pre-existing RNA. From the data obtained it is concluded that the newly synthesized RNA of the ribosome fraction in phage infected cells is indeed a phage-specific component. (Supported by grants from NIH, Nonr, and NSF.)

Biochemistry

QUANTITATIVE AND QUALITATIVE DIFFERENCES AMONG THYMIC RIBONUCLEASES. Patricia P. Weymouth (intr. by J. H. Pazur.) Univ. of Nebraska, Lincoln, Nebraska

Previous studies have suggested the presence of more than one RNase in mouse thymus. Seven fractions with RNase activity have now been obtained by elution of acid soluble thymic proteins from CM-cellulose. Activity in these fractions was measured, with RNA as substrate, as follows: (1) formation of acid soluble nucleotides; (2) rate of decrease in viscosity; (3) rate of decrease in optical density at 300 mμ. Six of the fractions could be grouped in order of increasing activity by assay (1) as follows: I, III, V; II, IV; and VII, with activity in VII 10 times that in the first group. By assay (2), there was an 80 fold increase in activity in the order; V, IV, III, I, II, and VII. Fractions I, II, and VII showed the same activity by assay (3). Further differences were seen in the pH optima for production of nucleotides and in paper electrophoretic behavior. The pH optima were: less than 5 for VI; 5-6 for III and VII; 6-7 for IV; and 7-8 for V, at μ 0.02 and 0.12, respectively. The patterns of electrophoretic migration of fractions V and VI and pancreatic RNase were different in direction or rate of movement. These results point to the occurrence of several RNases in the thymus. (Supported by Grant C4333(1) from the U. S. Public Health Service.)

Biochemistry

ENZYMATIC SYNTHESIS OF POLYNUCLEOTIDES CONTAINING THIOURIDYLIC ACID. Peter Lengyel and Robert Warner Chambers (intr. by S. Ochoa) New York University College of Medicine, New York.

In order to gain further insight into the biological role of polynucleotide phosphorylase, we have examined 2-thiouridine 5'-diphosphate (TUDP) as a substrate for this enzyme. If polynucleotide phosphorylase is involved in RNA biosynthesis, then TUDP must serve as a substrate for the enzyme in vitro, since it has been shown that thiouracil (TU) is incorporated into RNA in vivo. TUDP, prepared from uridine by an 8 step chemical synthesis, showed an activity similar to that of UDP in the P^{32} exchange assay for polynucleotide phosphorylase. Incubation of TUDP with the enzyme produced polythiouridylic acid (poly TU). The polymeric nature of the product is indicated by gel formation during synthesis and its high sedimentation coefficient (S_{20}=43) in the ultracentrifuge. These results suggest that poly TU exists as long, rather extended chains. An RNA-like polymer was synthesized enzymatically from a mixture of TUDP, UDP, CDP, ADP and GDP. These observations suggest that polynucleotide phosphorylase may be involved in the incorporation of TU into RNA in vivo. We are indebted to Dr. R.C. Warner for the ultracentrifugal data. (Supported by a grant from the National Science Foundation.)

Biochemistry

MOLAR BASE RATIOS OF NORMAL TISSUE, TUMOR, AND TISSUE SUBFRACTION RNA. Saul Kit. The Univ. of Texas M. D. Anderson Hospital and Tumor Institute, Houston, Texas.

The suggested roles of RNA as templates and carriers of coded information in biological systems has stimulated interest in the comparative composition of these polynucleotides from tissues differing in function and mitotic activity. RNA was extracted with aqueous phenol from 4 normal tissues of mice, livers of newborn mice, 7 tumors including diploid and tetraploid lymphomas, melanomas, and carcinomas. RNA was also prepared from the following subfractions of spleen and lymphoma tissues: 1) microsomes; 2) a particulate fraction extracted from the nucleus with 0.1M phosphate buffer and sedimented by centrifuging for 2 hours at 105,000 g; and 3) the Residual nuclear fraction. The molar purine and pyrimidine base ratios of the various tissues and tissue subfractions were measured after paper chromatography. The ratios of G+CRP/A+URP of the total RNA ranged from 1.78-1.96. The molar base ratios of the Total RNA of the various tissues were very similar. That of spleen and lymphoma microsomal RNA were similar, as were spleen and lymphoma nuclear particle RNA, or the Residual nuclear RNA of 4 lymphomas. For each tissue, the base composition of the nuclear particles resembled that of the microsomal particles or the total RNA. The Residual nuclear RNA did not significantly differ from the other fractions.

Biochemistry

ACTION OF RIBONUCLEASE ON POLYRIBOPHOSPHATE AND STUDIES ON A NEW POLYSUGARPHOSPHATE. E. Rosenberg* and S. Zamenhof. Dept. of Biochemistry, Columbia University, New York.

Polyribophosphate(PRP), the type-specific substance of Hemophilus influenzae type b (Zamenhof et al, J. Biol. Chem. 203:695, 1953; Fed. Proc. 13:327, 1954) is depolymerized by Worthington pancreatic ribonuclease and equally well by ribonuclease A even though PRP contains no pyrimidines. According to viscosity measurements, the depolymerization is 2500 times slower for PRP than for RNA at pH 7.3. A hypothesis is advanced that ribonuclease requires for its action the presence in the aglycon of a hydroxyl group with a specific spatial orientation to the ribose. Such a hydroxyl is present in pyrimidines of RNA and in PRP but not in purines. Type specific substance f was also isolated and studied (in collaboration with G. Leidy and E. Hahn). It is a polysugarphosphate free of purines and pyrimidines. On acid hydrolysis it yields galactosamine and phosphate; the sugars are connected by phosphate links (sugar/phosphate 2.01). Like PRP, substance f is a non-dialyzable viscous polymer unaffected by intestinal phosphatase but readily depolymerized by alkali; this treatment liberates secondary phosphates and makes the product susceptible to quantitative dephosphorylation by phosphatase. Even after extensive dialysis, substance f appears to contain reducing groups to the extent of 9% of sugars.

Biochemistry

EVIDENCE THAT 5'-NUCLEOTIDES ARE THE BUILDING BLOCKS FOR BIOSYNTHESIS OF RNA IN RAT LIVER. T. D. Price, R. A. Darmstadt, H. A. Hinds and K. K. Tsuboi (intr. by B. Weiss). College of Physicians and Surgeons, Columbia Univ., New York.

When fasted Long-Evans rats are injected with phosphate-P^{32}, sacrificed after 20-60 min., and the acid-soluble nucleoside-5'-monophosphates isolated from liver and evaluated, it is found that a very selective intracellular P^{32}-labeling has occurred naturally. At shortest time, relative molar specific activities of 5'-AMP, 5'-CMP, 5'-GMP and 5'-UMP were found to be 100, 12, 10 and 4.3 respectively. Carbon-bound phosphate groups of the nucleoside-5'- di- and triphosphates studied have labeling nearly equal to that of cognate monophosphates. The four nucleoside-5'-phosphate units of nuclear and cytoplasmic RNA are characterized by marked heterogeneity of labeling closely resembling that of the acid-soluble nucleoside-5'-monophosphates. In contrast, the four 3'-nucleotides from alkaline degradation of nuclear RNA are almost equally labeled. These findings point to a conclusion that nuclear RNA of rat liver is assembled from 5'-nucleotide building blocks in vivo. They are irreconcilable with postulated mechanisms involving synthesis of RNA from (i) 3'-nucleotides, or (ii) a general phosphate donor and nucleosides. 3'-CMP was 2-4 times more active than other 3'-nucleotides of cytoplasmic RNA, suggesting in vivo exchange of pA at pCpA ends of certain RNA molecules.

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Biochemistry

STRUCTURAL DETERMINANTS OF RNA. Warren E. C. Wacker, Keiichiro Fuwa and Robert Druyan, (Intr. by F. L. Hoch) Biophysics Research Laboratory of the Dept. of Med., Harvard Medical School and Peter Bent Brigham Hospital, Boston, Mass.

With rising temperature, RNA undergoes a phase-transition as evidenced by an increase of E_{258} and a decrease of $[\alpha]_D$ (Doty et al., N. Y. Acad. of Science 81, 693, 1959). RNA from various sources contains significant amounts of firmly bound metals. These metals, intrinsic to RNA, (Wacker and Vallee, J. Biol. Chem. 234,3257, 1959) are now shown to affect markedly both specific rotation and absorbance, indicating a possible function. For example, the melting temperature, (T_M), is lowered by partial removal of intrinsic metals and raised by the addition of chromium, nickel, iron, manganese and magnesium. They also alter the hydrodynamic properties of RNA, further suggesting that these metals contribute to the formation and preservation of its helical structure. In addition to effects on hydrogen bonding between bases, the stabilization of RNA as a function of temperature, brought about by the presence of metals, suggests cross linking of the bases through coordination or "sandwich" complexes as possible mechanisms underlying these changes.

8

Biochemistry

DENATURATION OF PEA MICROSOMAL RNA AND CALF THYMUS DNA IN FORMAMIDE AND DIMETHYLSULFOXIDE. George K. Helmkamp* and Paul O. P. Ts'o. Calif. Inst. of Technology, Pasadena, Calif.

In the search for non-aqueous systems for carrying out chemical modifications of RNA and DNA, it was found that formamide and dimethylsulfoxide were effective solvents. The secondary structures of these nucleic acids in organic solution were investigated. The $[\alpha]$ vs temp. profiles were in sharp contrast to those of aqueous solutions, for temp. had little effect on these weakly rotatory solutions. Thus, the nucleic acid appears to lose its intramolecular H-bonding and helical structure in the process of solvation. For verification, the nucleic acids were studied after their transfer to aqueous systems. Here, DNA showed a temp. dependence of optical density and rotation reminiscent of that of heat-denatured DNA (broad melting zone, little hysteresis). For RNA however, heat- or urea-denatured products have a profile similar to that of native RNA, so ultracentrifuge studies were used to identify denatured products. Native RNA consists of 28S and 18S components which on denaturation are modified into a broad boundary with an average S value of 7-11S. This pattern is duplicated by RNA with a history of solution in the above solvents, again indicating a denaturation effect. The optical properties of solutions of mixed nucleic acids are being studied in a search for possible interactions.

9

Biochemistry

REVERSIBLE INACTIVATION OF INFECTIOUS RNA BY RIBONUCLEASE H. Fraenkel-Conrat and B. Singer, Virus Laboratory, Univ. of California, Berkeley 4, California.

Evidence has been presented that infectious RNA can be isolated by the cold phenol method from animal tissue extracts containing no virus, or no cold-phenol-splitable virus (e.g. Wecker, Virology 7, 241, 1959). Similar findings with plant extracts are not regularly reproducible. However, a model system behaving similarly could be constructed by the addition of pancreatic ribonuclease to viral RNA. Best results were obtained by adding to .01% RNA at 0° in salt-free solution one thousandths (w/w) of RNase (0.15 mole enzyme/mole RNA). After varying time periods part of the solution, made 10^{-3}M pH 7 with Versenate, was extracted with 80% phenol. Assay of the original mixture showed no infectivity, at any time. In contrast phenol treatment after 15 minutes regenerated in average 20% of the original infectivity. Recovery decreased with time of contact, and was usually lower when greater or smaller amounts of RNase were used. In presence of salts very much less enzyme inactivates, largely irreversibly. Like RNase, a crude tobacco plant extract at the proper level was found to inactivate viral RNA in distilled water in partially reversible manner. It seems that a natural intracellular enzyme-associated state of the RNA may have biological significance, and may account for some of the above quoted observations.

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Biochemistry

Thermodynamic Aspects of RNA Synthesis. By P. George and R.J. Rutman, Harrison Lab. of Chemistry, Univ. of Penna.

When RNA reacts with adenosine diphosphate (ADP), the polynucleotide is lengthened by successive formation of 3'-5' diester phosphate linkages. Since K_{obs} at pH 7.5 is about unity, ΔF_{obs} for hydrolysis of the new 3'-ester linkage must be almost equal to that for the ADP anhydride. Calculation shows that the ΔF_{obs} values, -7.3 and -7.8 kcal/mole, contain similar RTlnH contributions, and $\Delta F'$ for the ionized species (standard state H=1) is +2.6 and -0.9 kcal/mole respectively. There are 'n+1' primary ionizations in reactant RNA, and 'n' in product RNA; their contributions to hydrolysis largely compensate, and the reaction systematically resembles the orthophosphate split of ADP since both release a nucleotide and another product which has two pK's operative in the pH region 0 to 7.5. ΔF^o (unionized species, H=1) is found to be -5.2 and -7.0 kcal/mole respectively, and ($\Delta F' - \Delta F^o$), the free energy increment due to participation of ionized species, has similar values, i.e., +7.8 and +6.1 kcal/mole. However, the correspondence of ΔF^o values has structural implications since it shows that insertion of R-O into the O-P-O-P-O anhydride group of ADP to give the O-P-O-R-O-P-O linkage in RNA, has had little effect on the reactivity.

11

Biochemistry

BASE INTERACTION BETWEEN OLIGONUCLEOTIDES AND LONG-CHAIN POLYMERS. Marie N. Lipsett (intr. by Leon Heppel). Natl. Insts. of Health, Bethesda, Md.

Ascending series of short-chain polynucleotides of adenylic and uridylic acids have been prepared by enzymatic degradation of the corresponding long-chain polymers (Poly A and Poly U, prepared with polynucleotide phosphorylase). Hydrogen bonding between Poly U and short polynucleotides of adenylic acid containing 2 to 10 base units has been observed by measuring hypochromicity. (Warner, R. C., Fed. Proc. 15, 379, 1956.) The converse interaction between Poly A and short uridylic acid polynucleotides was also found. The relative influences of different end groups and of oligonucleotide chain length on the strength of hydrogen bonding have also been investigated and will be reported.

12

Biochemistry

THE CONFIGURATIONAL TRANSITION OF TMV RNA. H. BOEDTKER. (intr. by P. Doty.) James B. Conant Laboratory, Harvard University, Cambridge, Mass.

The configuration of TMV RNA in solution was studied by observing the reversible transition from a partly ordered helical configuration, stable at room temperature in 0.1M sodium phosphate buffer, to a disordered extended one stable at high temperatures (above 70°) or in dilute salt solutions (less than 0.001M). The transition was observed optically by ultraviolet absorption measurements at 2600 Å, by optical rotary dispersion, and hydrodynamically by sedimentation studies. RNA samples prepared by the phenol method were relatively stable at 20° and up to 90% of the molecules sedimented with a single sedimentation constant in 0.1M sodium phosphate buffer ($S_{w,20}$ =28S). This sharp distribution of sedimentation constants was unaltered at lower salt concentrations although the mean S dropped to 3.3S. The change in sedimentation constant was found to occur over a narrow range of ionic strength and to parallel the change in UV absorption, even though some of the intermediary measurements were made in versene, or in phosphate buffer to which 10^{-4}M Mg had been added. Thus the RNA molecule undergoes a configurational transition at 20°, the molecule expanding dramatically as the secondary structure is disrupted. The absence of any evidence of configurational heterogeneity indicates that all the RNA molecules obtained from TMV have a very similar nucleotide composition and sequence.

13 *Biochemistry*

POLYNUCLEOTIDE PHOSPHORYLASE ACTIVATORS IN ADP PREPARATIONS. Roland F. Beers, Jr. Children's Hospital, Inc., Baltimore, Maryland.

Polynucleotide phosphorylase (polyase) activators have been isolated from commercial prepns. of ADP by ion exchange chromatography and are not artifacts of the procedure. They are adsorbed on Norite A charcoal, have max. absorption at 260 mμ and are presumably nucleotide derivatives. Their properties are similar to dialyzable activators obtained from RNA prepns. (R.F. Beers, Jr., Nature 183, 1335(1959)). The degree of activation increases from zero- to several-fold following treatment of B. subtilis polyase with charcoal. M. lysodeikticus polyase sensitivity increases with the 280mμ/260mμ ratio, but is marked with ratios as low as 1.0. A slight lag in polymerization of ADP is abolished by the activators. The latter are effective at concns. of 10^{-8}M. Preincubation of polyase with yeast RNA or poly A blocks the actions of the activators. Activation by yeast RNA or poly A at high salt concns. without preincubation is minimized by the activators. The activation may be a reversal of inhibition by polynucleotide contaminants in the enzyme prepns., a stimulation of primer synthesis, or both. The equilibrium of the polymerization reaction is not altered.

14 *Biochemistry*

ENZYMATIC INCORPORATION OF ATP AND CTP INTO POLYNUCLEOTIDES. Mary Edmonds* and Richard Abrams, Montefiore Hospital, Research Department, Pittsburgh 13, Pennsylvania.

A separation of enzymes responsible for the incorporation of ATP and CTP into polynucleotides has been achieved by fractionation of extracts of calf thymus nuclei with ammonium sulfate. 75% of the ATP enzyme and 10% of the CTP enzyme were found in one fraction while 80% of the CTP enzyme and 20% of the ATP enzyme were in another. A preparation of the ATP enzyme purified 100-fold incorporated 95% of the AMP of ATP into the interior of the polynucleotide chain. 99% of the P^{32} incorporated from $AMP^{32}PP$ was in 2',3'-AMP recovered from an alkaline hydrolysate of the polynucleotide. These data indicate the formation of a linear sequence of 25 to 100 AMP units. No primer requirement has been found. In contrast the CTP enzyme appears to require a primer as CTP incorporation stops after 10 min. This may be the enzyme in thymus tissue reported by Hurwitz, et al (Biochem.Biophys. Research Comm. 1, 3 (1959). However, terminal addition of CTP predominated in their preparations while it is 25% in these. Recovery of 75% of the incorporated P^{32} in CMP from an alkaline hydrolysate of polynucleotide labeled with $CMP^{32}PP$ suggests the formation of a sequence of CMP nucleotides. (Aided by grants from the American Cancer Society and the U. S. Atomic Energy Commission).

15 *Biochemistry*

CHROMATOGRAPHIC AND METABOLIC BEHAVIOR OF YEAST RNA FRACTIONS. Jason Starr* and David Goldthwait, Dept. of Biochem., Western Reserve U., Cleve., O.

Two different RNA fractions from yeast have been characterized chromatographically. They can be isolated either by differential solubility in NaCl or by ultracentrifugation. 80% of the RNA is insoluble in NaCl or can be isolated in a microsomal fraction. It is eluted from Ecteola at pH 9.5, has an S_{20w} of 4.5, and does not stimulate C^{14} leucine incorporation. 20% is soluble in 1 N NaCl or can be isolated in the 105,000 X g supernatant. It is eluted from Ecteola with neutral NaCl, has an S_{20w} of 3.2, and stimulates C^{14} leucine incorporation. The terminal nucleosides after alkali digestion are 62% adenosine and 38% uridine. An $(NH_4)_2SO_4$ fraction of a rabbit muscle extract catalyzes the incorporation of ATP^{32} into the terminal nucleotide of this RNA, 90% next to CMP. Incorporation proceeds linearly for 1 hr up to 25 mμm/mg of RNA. After the RNA is incubated with CTP for 1 hr, analysis of the end groups shows that one-half of the adenosine is replaced by cytidine.

16 *Biochemistry*

INCORPORATION OF ATP-C^{14} INTO POLYRIBONUCLEOTIDES. J. Preiss* and P. Berg. Stanford University School of Medicine, Palo Alto, California.

A partially purified enzyme fraction from E. coli catalyzes the incorporation of ATP-C^{14} into an acid-insoluble product. This conversion is not inhibited by $4x10^{-2}$ M inorganic phosphate and is markedly stimulated by a triphosphate-generating system. The radioactivity of the product is made completely acid-soluble (95 percent or better) by treatment with RNase or 0.3 N NaOH for 20 hours at 37° but no degradation occurs after exposure to DNase (3 μg). With this enzyme fraction the incorporation of ATP-C^{14} is almost completely dependent upon addition of RNA. While the purified amino acid-acceptor RNA of E. coli is active, exposure of the acceptor RNA to purified venom phosphodiesterase (5 percent hydrolysis) increases its activity 8-10 fold. With various preparations there is about a 2-5 fold acceleration in the rate of ATP-C^{14} incorporation by the addition of CTP while other triphosphates have little or no effect. Alkaline degradation of the product formed from ATP-C^{14} yields 95 percent of the radioactivity as adenosine suggesting that the incorporated nucleotide is probably terminal.

17 *Biochemistry*

PREPARATION AND PROPERTIES OF POLYRIBOGUANYLIC ACID. Sanae Mii* and Robert C. Warner, Dept. of Biochemistry, New York Univ. College of Medicine, New York, N. Y.

Although polynucleotide phosphorylase catalyzes the exchange of the terminal phosphate of GDP with Pi and GDP is readily incorporated together with ABP, UDP and CDP into a copolymer, no homopolymers of guanylic acid (poly G) with long chain lengths have been made. The synthesis of poly G has been reinvestigated using highly purified enzyme and synthetic GDP. Using a higher ratio of enzyme to nucleotide than previously employed the reaction proceeded to 45% liberation of Pi. The rate was increased and the Pi liberation reached 75% when poly C was used as a primer. Copolymers of guanylic and cytidylic acids have also been made. The poly G thus prepared exhibited a hypochromic effect of 32%. It was highly aggregated in neutral solution and sedimentation coefficients (S_{20}) up to 17 have been observed. On standing it gradually precipitates. When redissolved above pH 11 the S_{20} dropped to 2.5. Some hydrolysis at this pH is not excluded although poly G is more resistant to alkaline hydrolysis than other polynucleotides. The same preparation had a chain length greater than 100 nucleotide units as estimated from end group determinations. Complexes, similar to those previously obtained between poly A and poly U, are formed when poly G and poly C are mixed as shown by changes in the UV absorption and S_{20}.

18 *Biochemistry*

AMINO ACID AND RNA METABOLISM IN CHLORAMPHENICOL TREATED L. CASEI. C.E.Carter. Yale Univ. School of Med., New Haven, Conn.

Cell suspensions of L. casei treated with chloramphenicol accumulated RNA in the 100,000 x g supernatant fraction. Compared with normal cells the chloramphenicol - SRNA had a higher guanine content and specific activity when guanine-C^{14} was present in the media. The acid-soluble fraction of chloramphenicol treated cells contained 3 times more guanosine nucleotides than the control. In media completely supplemented with amino acids the assimilation of guanine-C^{14} into the RNA of treated and normal cells reached a plateau at 2 hours of incubation. When tryptophane and phenylalanine were omitted from the media, radioactivity of the RNA decreased after 2 hours in normal cells but continued to increase in chloramphenicol treated cells and exceeded the level found for complete media. The omission of lysine did not alter the rate of guanine assimilation during the first hour of incubation of treated and normal cells but subsequently the amount and radioactivity of RNA decreased in both. Orotic acid-C^{14} and guanine-C^{14} were compared as RNA precursors in complete and aromatic amino acid deficient media. In the presence of chloramphenicol, cells incubated with guanine-C^{14} showed a 200 per cent greater incorporation of radioactivity into RNA in the aromatic amino-acid deficient than in complete media; replicate experiments with orotic acid-C^{14} showed a 50 per cent increase under similar conditions.

XUM

19 *Biochemistry*

STUDIES ON S-RNA SYNTHESIS: FRACTIONATION OF THE ENZYME-RNA COMPLEX. Edward Herbert* and E.S.Canellakis. Dep't of Biology, Mass. Inst. Tech., Boston, Mass. and Dep't of Pharmacology, Yale Univ. Med. School, New Haven, Conn.

Rat liver contains an enzyme complex which incorporates ribonucleotides into terminal positions of the soluble ribonucleic acid (S-RNA). This enzyme complex has been purified several hundred-fold to yield three ribonucleoprotein fractions, designated Ribonucleoproteins α, β and γ. Two of these, Ribonucleoproteins β and γ, contain the bulk of the original enzymatic activity in incorporating C^{14}-ATP and C^{14}-CTP into terminal positions of the S-RNA. The best preparations of the Ribonucleoproteins β and γ have $E_{260}:E_{280}$ ratios of 1.7-1.9 (in 0.1M PO_4^-, pH 7.2) and retain full enzymatic activity for periods up to 8 months, when stored as lyophilized powders. These preparations have been separated into a predominantly protein component ($E_{260}:E_{280}$=0.65-0.70) and a predominantly RNA component ($E_{260}:E_{280}$=2.01-2.10). When incubated alone, neither component shows any of the original enzymatic activity; full enzymatic activity is restored when the protein and the RNA component are incubated together. Ethanol precipitation of the RNA component does not affect its activity in the enzymatic assay, but phenol extraction results in a decrease of activity. Low activity is obtained when the phenol extracted S-RNA from the pH 5 fraction is combined with the protein component.

20 *Biochemistry*

ACTION OF MICROCOCCAL NUCLEASE ON OLIGONUCLEOTIDES. W. Roberts* and C. Dekker. Dept. of Biochem., Univ. of Calif., Berkeley.

Micrococcal nuclease was shown by J.D. Smith (unpublished) and subsequently by K.K. Reddi (Biochem. et Biophys. Acta, 36, 132(1959)) to hydrolyze poly A, poly C and poly U giving only 3'-phosphate terminated mono- and dinucleotides in ratios varying from 3:1 to 6:1. Such a product distribution is best explained as resulting from initial endoesterase action on the polymer followed by exoesterase action on the oligomers produced, the stepwise removal of nucleoside-3'-phosphate units terminating when a dinucleotide remains. To determine the end from which the mononucleotide units are removed we have studied the action of the 500-fold purified enzyme (M.L. Dirksen, unpublished) on ApApCp, ApApUp and GpGpUp. The products were ApAp or GpGp and the pyrimidine containing mononucleotides. This would indicate that the exoesterase action proceeds from the end bearing the 3'-monoesterified phosphate, a mode of action different from that suggested by Reddi. Fragments lacking the terminal phosphate, e.g. ApApC and ApApU, are hydrolysed at either phosphodiester linkage and the products so formed (ApAp, ApC or ApU) resist further hydrolysis. Although the possibility that we are dealing with two or more enzymes cannot be excluded, the above results, as well as additional results using polydeoxyribonucleotides as substrates, suggest a mechanism which would account for both endo- and exo- activity by one enzyme.

21 *Biochemistry*

PURIFICATION, PROPERTIES AND MECHANISM OF ACTION OF RIBONUCLEASE FROM E. COLI RIBONUCLEOPROTEIN PARTICLES. P. F. Spahr and B. R. Hollingworth (intr. by J. T. Edsall). Biological Laboratories, Harvard University, Cambridge, Massachusetts.

E. Coli ribonucleoprotein particles (s_{20}=70 S), (A.Tissieres et al., J. Mol. Biol. in press) contain a latent ribonuclease which can be liberated by treatment with 4 M urea (Elson, Biochim. Biophys. Acta 27, 216 (1958); Bolton and McCarthy (Biophys. Soc. Abstracts, P9 (1959)). It has been isolated by treatment with 4 M urea, ammonium sulfate fractionation and chromatography on Amberlite IRC 50. An acid phosphatase is also present and is resolved from ribonuclease in this chromatographic step. The purified ribonuclease is free of phosphatase and shows no DNase activity. It does not act upon Ca[bis(paranitrophenyl)phosphate]$_2$. Its optimum pH lies at pH 8.0 when assayed on yeast ribonucleic acid. When purified the enzyme is unstable in dilute solution and can be stabilized by addition of dilute serum albumin. The enzyme cleaves all internucleotide linkages in RNA from 70 S particles giving rise to 2':3' cyclic nucleotides which in turn are converted to the corresponding 3' nucleotides. Dialysable fragments released during initial stages of digestion are rich in cytosine and poor in adenine. The enzyme splits the 6-amino cyclic nucleotides much faster than the 6-keto.

22 *Biochemistry*

The Incorporation of Ribonucleotides into RNA
M. Alexander*, A. Bresler*, J. Furth* and J. Hurwitz Dept. of Microbiology, N.Y. Univ., N.Y.,N.Y. In the presence of CTP, Mg^{++} and RNA, CMP is incorporated into RNA according to the equation RNA+CTP⟶RNA-CMP +PP. It has been found that for every mole of CMP incorporated, one equivalent of PP is formed. The RNA required in this reaction is obtained from thymus and is quite heterogeneous with an average sedimentation constant of approximately 4. Attempts to replace this RNA with liver RNA poly A, poly C and a variety of other RNA preparations have been negative. RNA derived from E. coli will partially (10%) replace thymus RNA. The enzyme system catalyzing the above reaction has been purified 100 fold from thymus. It can be replaced by a partially purified enzyme fraction derived from E.coli suggesting that the RNA primer requirement is not species specific. The activity with the bacterial preparation is not due to polynucleotide phosphorylase activity since this system has a high affinity (K_s<10^{-5}M) for CTP and is insensitive to Pi addition. (Grant E-2869, N.I.H.)

23 *Pharmacology*

THE AMINO ACID ACCEPTOR RNA IN MICROSOMES AND ITS RELATION TO PROTEIN SYNTHESIS. Kendric C. Smith and Ruth G. Doell (intr. by Robert H. Dreisbach.) Stanford Univ. Sch. of Med., Palo Alto, California.

In mouse liver microsomes there is a fraction of RNA comprising 10-20% of the total RNA which will accept amino acids (54% as active as soluble RNA) when incubated with pH 5 enzymes under the usual conditions. This RNA cannot be removed from the microsomes by 3 washings in 0.25M sucrose-0.001 M $MgCl_2$. This RNA has been separated from the bulk of the microsomal RNA using the starch exchanger, CATO-2, which has been shown to separate tyrosine acceptor RNA from leucine acceptor RNA (Smith, Cordes and Schweet, Biochim. Biophys. Acta, 33; 286, 1959). When eluted from CATO-2 columns with 0.15M, 0.20M, and 1.0M NaCl; and with 1.0M NaCl-1.0M NH_4OH; soluble RNA shows the following distribution: 69%; 11%; 9% and 12%, respectively, while microsomal RNA shows a distribution of 1%; 8%; 12% and 80%, respectively. Of the various types of soluble RNA only those eluted by salt concentrations greater than 0.15M (30% of the total) are found in the microsomes. The RNA in these peaks has been investigated with respect to chemical composition (particularly the 5-ribosyl uracil content); relative in vivo labeling with C^{14} orotic acid; and ability to accept amino acids under various conditions.

24 *Pharmacology*

EFFECT OF 5-BIS(2-CHLOROETHYL) AMINOURACIL ON INCORPORATION OF URACIL-2-C^{14} INTO RNA IN TUMOR-BEARING RATS. Paul Byvoet* and Harris Busch. Dept. of Pharmacol., Univ. of Ill., Coll. of Med., Chicago, Ill.

The present experiments are an extension of studies on effect of mustards on nuclear metabolism of the Walker 256 tumor (Busch, Amer and Nyhan, J. Pharm. 127, 195, 1959). One hour after I.P. injection of 25 uc of uracil-2-C^{14} into tumor-bearing rats, the S.A. of RNA of the nucleus, microsomes and cytoplasmic sap of the Walker 256 carcinosarcoma were 122, 25 and 39 cpm/mg, respectively. In spleen, the S.A. of RNA of the nucleus, microsomes and cytoplasmic sap were 27, 27 and 13 cpm/mg, respectively. In liver, the S.A. of the RNA of nucleus and microsomes were 13 and 5, respectively. Thus, incorporation of uracil-2-C^{14} into nuclear RNA in the tumor was 4-5 times that of the microsomal RNA, while in spleen and liver, incorporation of uracil-2-C^{14} was 1-1.5 times that of microsomal RNA. The S.A. of nuclear RNA of the tumor was 5 and 9 times that of spleen and liver, respectively. At 24 hrs following I.P. administration of 4 mg/kg, the aminouracil mustard produced a uniform 20-25% suppression of incorporation of uracil-2-C^{14} into RNA of the tumor, complete suppression of uptake into RNA of spleen and a 2-3 fold stimulation of uptake into RNA of the nucleus and microsomes of the liver.

203. NUTRITION

Nutrition

PRECURSORS OF SHORT-CHAIN FATTY ACIDS IN THE RUMEN OF SHEEP FED PURIFIED DIETS. Darrell Van Campen* and Gennard Matrone. Nutrition Sec., North Carolina State College, Raleigh.

Studies were conducted on the in vivo and in vitro metabolism of C^{14}-labeled bicarbonate and acetate-2-C^{14} by rumen microorganisms of sheep receiving two purified diets, i.e. Diet 1 and Diet 1(Na+K) as described by Matrone et al. (Proc. Soc. Exp. Biol. Med. 100:8, 1959). A major portion of the label in the C^{14}-bicarbonate studies was accounted for in the propionate and valerate fractions. It was also observed that the rumen microorganisms from the sheep receiving dietary sodium and potassium bicarbonate, Diet 1(Na+K), showed a higher, in vitro, incorporation of labeled carbon dioxide than cells from the sheep fed Diet 1. The majority of the incorporated label in the acetate experiments could be accounted for in the butyrate and valerate while much smaller amounts were found in propionate. The in vivo studies suggested a product-precursor type relationship between acetate and butyrate. In the in vitro studies with acetate-2-C^{14}, the presence of sodium and potassium bicarbonates appeared to stimulate production and labeling of butyrate and valerate. (Supported in part with grants from the Herman Frasch Foundation and E. I. duPont de Nemours and Company, Wilmington, Delaware.)

Nutrition

VITAMIN K REQUIREMENT OF THE RAT WHEN FED IRRADIATED BEEF. William S. Worth, Merrill S. Read and Richard W. Pospistle (intro. by H. E. Sauberlich.) U. S. Army Medical Research & Nutrition Lab., Denver 30, Colorado.

A gradient evaluation of the rat's requirement for vitamin K_1 or K_3 was studied while fed an irradiated beef diet. Male (Sprague Dawley) weanling albino rats were fed a vitamin K deficient hemorrhage-producing diet containing 35% irradiated (5.58 megarad) beef (Metta, V.C. et al., J. Nutr. 69, 18-22, 1 Sept. '59) for 70 days. Menadione, Synkayvite (sodium menadiol diphosphate) or vitamin K_1 was administered in three separate studies. Daily oral levels were 0.25, 0.50, 1.0, 2.0 or 4.0 μgm in each study. Blood clotting potentials were determined by a semi-micro modification of the Quick one-stage prothrombin test. Growth curves were essentially the same within and between the three studies. Prothrombin values approached normal as the amount of vitamin K administered increased. Upon death, typical diathetic hemorrhage was observed in the cranial cavity, thymus, cervical lymph nodes, retroperitoneal area and/or urogenital organs. Rats required 2 μgm menadione, 8 μgm Synkayvite (based on menadione equivalence) or 2 μgm vitamin K_1 to prevent death due to hemorrhagic diathesis. However, to sustain normal prothrombin values, 4 μgm menadione, 8 μgm Synkayvite or 3 μgm vitamin K_1 were required daily.

Nutrition

SINGLE AND COMBINED ADDITIONS OF ARSANILIC ACID, FURAZOLIDONE AND RESERPINE IN A LAYING HEN DIET. C. W. Carlson, South Dakota State College, Brookings, South Dakota.

Dietary additions of arsanilic acid and furazolidone resulted in some improvements in egg production (Carlson 1959, Tech. Bul. 22, So. Dak. Agric. Exp. Sta.), whereas the tranquilizer reserpine was shown to relieve stress conditions (Van Matre, et al. 1958, Poultry Sci. 36: 1164). With SCWL pullets in floor pens, (60 per pen, replicated) one strain with a higher rate of mortality showed a marked egg production and feed efficiency response to both arsanilic acid (45 mg/lb) and furazolidone (7.5 mg/lb) whereas the other strain showed little response. With similar stock in individual cages, (15 per group, replicated) only the combination of arsanilic acid and furazolidone showed an improvement in egg production. Pullets receiving reserpine (0.50 mg/lb., used only for caged stock) showed a small positive response in egg production with the control diet or diets containing either additive alone, but not the combination. A slight reduction in blood cholestrol level was obtained with reserpine, whereas no consistent effects were noted on egg weight, shell thickness, Haugh unit value, or yolk color. Hatchability of eggs produced by hens in the floor pens was not affected by diet. Growth rate of progeny from the responsive strain was superior from hens receiving the combined additives only.

Nutrition

REPRODUCTION STUDIES IN THE MATURE FOWL AS AFFECTED BY CORN, SOYBEAN OIL MEAL, CORN STEEPWATER AND CORN ASH. J. R. Couch, C. R. Creger* and R. E. Davies*. Tex. Agri. Expt. Sta., College Station, Texas.

Day-old pullet chicks are randomly divided into 1 group of 150 birds and 3 groups of 20 birds and fed a purified diet, and corn, soybean oil meal, and a combination with corn and soybean oil meal at the expense of glucose monohydrate and soybean protein respectively to maturity. The birds in group 1 were randomly distributed into 7 groups of 18 birds at 27 weeks of age and were fed the semi-purified diet supplemented with 25% corn, fractions of corn, corn steepwater and corn ash for a period of 12 weeks. The remaining groups were continued on the diets first mentioned. Substitution of corn, soybean oil meal, or corn steepwater into the purified diet on an isocaloric and isonitrogenous basis increased egg production. The fractionation or ashing of the corn and feeding of same had no effect on egg production. Substitution of corn, soybean oil meal, corn ash, or corn steepwater into the basal diet increased the hatchability of fertile eggs.

Nutrition

ALGAE FEEDING IN HUMANS: ACCEPTABILITY, DIGESTIBILITY AND TOXICITY. **M. E. McDowell, R. C. Powell, E. M. Nevels, J. H. Sellars and N. F. Witt** (intr. by John B. Youmans). U.S. Army Medical Research and Nutrition Laboratory, Denver, Colorado.

Algae may provide food for space travel and for protein supplementation. Chlorella ellipsoidea, high in protein (59%) generously furnished by Japan Nutrition Assoc., Tokyo, was fed to 4 normal men in increasing amounts in a diet constant in calories, in the following schedule: 10 grams algae per day for 6 days; a free day; 20 g/day for 6 days; free day; 50 g/day for 6 days; free day; 100 g/day for 3 days; 4 free days; 200 g/day for 3 days; 500 g (attempted) per day for 2 days. Mild gastrointestinal symptoms occurring chiefly on the first day of each period led us to omit "free days" between the last 2 periods. Algae mixed in food was reasonably palatable at 100 g/day. At 200 g/day it was tolerated but with definite distaste and more gastrointestinal symptoms. 2 subjects successfully ingested 500 g/day for 2 days, the others tolerating only 375 g, 61 g and 175 g, 300 g. All developed nausea, fullness, abdominal aching or cramps, mild malaise and headache. No diarrhea occurred; stools became dark green, bulky, formed, and were frequently dry and hard. Physical examinations and clinical laboratory tests failed to reveal toxicity.

Nutrition

THE EFFECT OF MICROBIAL CONTAMINATION ON THE REQUIREMENT OF CHICKS FOR CERTAIN NUTRIENTS. **Hardy M. Edwards and Frank M. Boyd.*** U. of Georgia, Athens.

The effect of environmental microflora on the quantitative requirement of nutrients by animals has received little attention. Chicks reared in a cleaned and fumigated animal laboratory were found to have a lower requirement for certain nutrients (magnesium and zinc) than chicks reared in a contaminated laboratory. By making use of isolation rooms, it was found that the effect of contaminated animal quarters could be simulated by feeding feed containing 1/2 or 1% chicken feces or by administering orally a water extract of feces. Using this technique, the chicken's requirement for magnesium and phosphorus was found to be higher under contaminated conditions. This higher requirement for phosphorus was evident when growth was the criterion; when per cent bone ash was the criterion, no difference in phosphorus requirement was noted. The magnesium requirement of chicks inoculated with Salmonella gallinarum was studied. The data obtained indicate that the magnesium requirement of the inoculated chickens may be higher, however, the requirement for magnesium is not increased as much as when chicken feces are fed.

7 *Nutrition*

BONE DENSITY AND CALCIUM INGESTION. Herald Schraer and Wm. John Sier. Dept. of Physics, Pennsylvania State Univ., Univ. Park, Pa. (intr. by I. Zipkin. Natl. Insts. of Health, Bethesda, Md.).

The utility of quantitative radiography as a tool for investigating calcium nutrition in vivo was studied. Male rats 35 days old were divided into 4 groups of 7 animals per group. Each group received a basal low calcium diet with calcium supplements added as $CaHPO_4$ and $CaCO_3$ in the ratio of 2.0: 0.75. Groups 1-4 respectively received calcium in the diet in the following percentages (by analysis): 0.03, 0.17, 0.9 and 1.5. All animals were quantitatively radiographed at the initiation of the experiment and on days 10, 17, 38, 45, 52 and 59. On day 52, the 0.03% Ca group was changed to the 0.9% level. Calcium balances on all animals were run for days 10-17, 38-45 and 52-59. Film densitometry across the femur diaphysis and distal epiphysis, and the seventh caudal vertebra revealed that the 0.9 and the 1.5% Ca groups had the same mean density values throughout. The 0.17% Ca group values were significantly lower. The 0.03% Ca group values were the lowest. In this group, the increased Ca intake on day 52 was followed by a sharp rise in density values clearly detectable within 4 days. Ca retention and bone density change were positively correlated. (Supported by research grant A-1292 M and N, NIH.)

8 *Nutrition*

EFFECT OF DIETARY VITAMIN E, FAT AND IRON ON Fe^{59} METABOLISM IN RATS. R. H. Mayo,* R. G. Buescher* and M. C. Bell. UT-AEC Agric. Res. Lab., Oak Ridge, Tenn.

Three experiments were conducted using semipurified (casein-corn starch) diets low in iron and Vitamin E. In all experiments rats were dosed orally with 5 μc of Fe^{59} and sacrificed after 96 hours to determine tissue activity. In two seven-week experiments (2 x 2 factorials), 50 gm. rats were fed two levels of added iron, 4 and 16 ppm (exp. 1) or 5 and 100 ppm (exp. 2) and 55.5 or 555 mg. of dl-α-tocopheryl succinate per kg. of diet. In Exp. 1 Vitamin E increased ($P < .05$) Fe^{59} levels in heart, kidney and spleen tissue but dietary iron had little effect. The 100 ppm of iron in Exp. 2 significantly lowered Fe^{59} in heart, liver, kidney, spleen and muscle tissue but increased the activity in blood, while Vitamin E had no significant effect. In Exp. 3, Fe^{59} tissue levels of Vitamin E deficient females fed 4 and 10% stripped lard were compared with ones fed 10% lard + 555 mg. of dl-α-tocopheryl succinate/Kg. diet. E-deficient rats fed 10% fat had significantly higher Fe^{59} activity in blood, spleen, kidney and muscle tissue than the other two groups. Tissue uptake of Fe^{59} was influenced by dietary Vitamin E, iron and fat.

9 *Nutrition*

OPTIMUM LEVELS OF AMINO ACID SUPPLEMENTATION FOR LIME-TREATED CORN. Ricardo Bressani. Institute of Nutrition of Central America and Panama (INCAP), Guatemala, C. A.

The number of limiting amino acids in lime-treated corn (masa) and optimum levels of supplementation were estimated from the growth, total serum protein and liver fat of rats. Groups of six animals were fed a basal masa diet supplemented with all nutrients, and varying levels of known limiting amino acids were added. The optimum amounts of added lysine and tryptophan in combination were 0.31 and 0.05%. For lysine alone 0.31% was also optimum but tryptophan alone had no effect. Lysine addition did not always increase growth, but consistently improved feed and protein efficiencies, raised serum proteins and lowered liver fat. When isoleucine, threonine and methionine were each tested at three levels in the presence of optimum lysine and tryptophan, the most desirable levels were 0.20, 0.20 and 0.15% respectively. Anyone of these three amino acids combined with lysine and tryptophan increased growth, feed and protein efficiency and serum proteins, and either threonine or methionine lowered liver fat. Methionine had the least effect in raising serum proteins. Two of the three also improved some or all of these measures. It is concluded that in rats the simultaneous addition of 0.31% L-lysine, HCl 0.5% DL-tryptophan and 0.20% DL-isoleucine, 0.20% DL-threonine and 0.15% DL-methionine to a corn masa diet is optimal as judged by the criteria used.

10 *Nutrition*

A LOW-COST PROTEIN RICH VEGETABLE MIXTURE FOR HUMAN CONSUMPTION. Nevin S. Scrimshaw, Ricardo Bressani, Moisés Béhar*, Dorothy Wilson* and Guillermo Arroyave. Institute of Nutrition of Central America and Panama (INCAP), Guatemala, C. A.

In continuing efforts to reduce protein malnutrition in technically underdeveloped areas, INCAP vegetable mixture 9 containing 27% protein and composed of 50% whole ground corn and sorghum, 30% cottonseed flour, 3% Torula yeast, 1% $CaCO_3$ and Vitamin A, has been subjected to extensive study. It produces satisfactory growth in chicks and young rats, good repletion and reproduction in adult rats and high Nitrogen Balance Index in puppies. In nitrogen balance studies in 10 children at intakes from 0.5 to 3.0 g of protein per kg, retentions were comparable to those with milk in alternate balance periods. Mixture 9 has been used successfully as the sole protein source in the initial therapy of 5 cases of Kwashiorkor and produces a serum amino acid response to a test meal similar to diets rich in animal protein. In field acceptability trials in 151 children in 4 localities in Guatemala, 93% liked the product and 81% consumed 2 or more glasses daily throughout the 4-month period. When placed on experimental sale with support of the local health center in a town of approximately 4,000 population, purchases during the initial 4 months have been equivalent to 3100 glasses per week, each containing 7 g of high quality protein at a cost of one cent.

11 *Nutrition*

EFFECT OF ARSENIC ON THE GROWTH OF MAMMALIAN CELLS IN VITRO. W. B. Savchuck, S. S. Schiaffino, and H. W. Loy (intr. by O. L. Kline.) Food & Drug Administration, Washington, D. C.

Growth of cells in tissue culture may be used as a means of studying physiological effects of compounds that are offered as food additives or as insecticides. Reaction at the cellular level may give important guidance to further metabolic studies. Application of this technique has been made in the studies of a series of arsenic compounds some of which are widely used in animal feeds. The growth of 4 species of cells (human liver, skin, HeLa, and "L" fibroblast) assayed by the DNA method showed that pycnosis and complete suppression of growth occurred at a concentration of pentavalent arsenic which was 10 to 30 times that of the trivalent form. Except for skin cells at low concentrations of pentavalent arsenic, stimulation of growth was not apparent in any of the test cultures. Studies evaluating the inhibiting effect of various organic arsenicals will be reported.

12 *Nutrition*

MICROBIOLOGICAL TECHNIQUES IN THE STUDY OF ARSENICALS. S. S. Schiaffino, H. W. Loy and W. B. Savchuck (intr. by O. L. Kline.) Food & Drug Administration, Washington, D. C.

Microbiological assay techniques may offer the advantage of sensitivity in measuring micro amounts of many compounds that appear in foods. In addition to the growth stimulating effect of many nutrients, we may also detect small amounts of growth-inhibiting substances such as insecticide or food additive residues in animal tissues. The effect of a variety of compounds on the growth of 30 microorganisms using tube or plate assay techniques was studied. It was observed that with L.leichmannii ATCC 7830, using the AOAC cyanocobalamin medium, growth was inhibited by the inorganic forms of tri- and pentavalent arsenic. Trivalent arsenic was found to be a more potent inhibitor of growth than was the pentavalent form. Detectable and reproducible inhibition of growth can be obtained at levels of 0.05 ppmof arsenic. Several organic arsenicals have been assayed with this technique. It was noted that trivalent organic arsenic was about one fifth as inhibiting as trivalent inorganic arsenic, whereas the difference in inhibition between the inorganic and organic forms of pentavalent arsenic was not as great. The results of this study present a new approach in evaluating the possible physiological effects of many substances used as food additives.

/3 *Immunology*

EFFECT OF REPEATED URINARY EXCRETION TEST ON B_{12} ABSORPTION & EXCRETION IN RATS. Victor Tantengco*, Kumio Okuda* and Bacon F. Chow. Johns Hopkins Univ., Sch. of Hyg. & Pub. Health, Balto.

The urinary excretion test is useful for the measurement of absorption of vit. B_{12} in man. Upon the repeated use of the same subject, the "carry over" of the residual radioactivity and the alteration of the state of "B_{12} saturation" due to previous inj. of the flushing dose may interfere with subsequent test. We therefore studied the effect of pre-inj. of B_{12} to rats on absorption and urinary excretion. Adult male rats were divided into 2 groups. Group A(8) received no injection and group B(9) were pre-inj. with unlabeled B_{12}, 20 ug for 2 days. On the 3rd day, ea. rat was admin. orally 10 mug B_{12} and inj. subcutaneously 50 ug of unlabeled B_{12} 2 and 24 hrs. afterwards. The radioactivity in 48 hrs. urine and fecal samples were measured. The same procedure was repeated 6 times with varying intervals. It was found that (a) in group A radioactivity in the urine increased from 6.3% to 11%, the amt. absorbed as measured by fecal excretion, decreased from 57% to 40%, (b) animals in group B absorbed less B_{12} on the 1st test and even less with subsequent tests. These data therefore demonstrate the unsuitability of using the same animal for repeated urinary excretion tests within short intervals of time.

204. NUTRITION–Carbohydrates

/ *Nutrition*

CELLULOSE DIGESTION BY THE RAT, A NONRUMINANT. R. Bernal Johnson and Donald A. Peterson (intr. by H. E. Sauberlich.) U. S. Army Medical Research & Nutrition Lab., Denver 30, Colo.

An isotope technique involving Carbon-14 has been used to investigate the digestion of cellulose by the rat. Tobacco and soybean cellulose C^{14} were fed and the Carbon-14 content of the rat's breath, urine, feces and carcass was determined. Digestion of tobacco cellulose C^{14} resulted in the following Carbon-14 excretion pattern: breath - 25%; feces - 58%; urine-1%; carcass - 8%. Digestion of soybean cellulose C^{14} resulted in a similar Carbon-14 excretion pattern: breath - 25%; feces-58%; urine - 1%; carcass - 6%. It appears that tobacco and soybean cellulose are digested equally well by the rat. Experimentation has shown that the gastrointestinal (G.I.)micropopulation of the rat is antibiotic sensitive and is essential for the digestion of cellulose. The G.I. micropopulation of the rat, which is moderately reduced by sulfasuxidine or sulfasuxidine plus penicillin and streptomycin, is markedly reduced by oxytetracycline. Oxytetracycline, which reduced the fecal bacterial colony count from $7 \times 10^6/cm^2$ to $2.2 \times 10^2/cm^2$ caused a markedly altered Carbon-14 excretion pattern: breath - 2%; feces - 95%; urine - 0.1%; carcass - 7%. Studies of cellulose metabolism by cecectomized rats will be discussed.

The cooperation of Dr. B. M. Tolbert, University of Colorado, is gratefully acknowledged.

2 *Nutrition*

STUDIES ON THE FERMENTATION OF ALFALFA HAY BY RUMEN MICROORGANISMS IN VITRO. CARBON DIOXIDE, METHANE AND HEAT PRODUCTION. T. V. Hershberger and E. W. Hartsook (intr. by R. W. Swift) Pennsylvania State University, University Park, Pa.

A direct, experimental evaluation of the efficiency of rumen fermentation has long been desired. To evaluate this process, the heat, CO_2 and CH_4 produced during a 24- or 32-hour in vitro fermentation of 10.8 Kgm alfalfa hay by ovine rumen microorganisms in 360 liters of rumen fermentation medium was determined utilizing the Armsby Respiration Calorimeter. Average results for 3 experiments indicated a heat production of 71.9 Calories and CO_2 and CH_4 productions of 117 gm and 12 gm, respectively, per Kgm alfalfa hay per 24 hours. In a typical experiment, the initial productions of heat, CO_2 and CH_4 increased rapidly, reached maxima at 5, 9 and 11 hours, respectively, and then decreased linearly thereafter. Molar ratios (CO_2:CH_4) decreased from 7.5 at 3 hours to 4 at 11 hours and remained essentially constant thereafter. It is concluded that the heat of rumen fermentation is quite small, less than 2%, and that the Armsby Respiration Calorimeter may properly be employed to study the energy relationships associated with the digestion of feeds by rumen microorganisms.

205. NUTRITION–Deficiency Diseases

/ *Biochemistry*

THE RELATIONSHIP BETWEEN VITAMIN DEFICIENCY AND CERTAIN BLOOD ENZYMES IN RATS. Myron Brin and Barbara Owens.* Departments of Biochem. & Medicine, State Univ. of N. Y. Med. Coll., Syracuse.

We recognize that certain enzymes in blood which require vitamin cofactors are significantly depressed in activity in deficiency states of those vitamins. In particular, erythrocyte transketolase is depressed in thiamine deficiency, and serum transaminases are depressed in pyridoxine deficiency.

Studies were designed to reveal the sensitivity of the enzyme response to a limiting vitamin. On diets, otherwise adequate, in which the concentration of thiamine was varied between zero and 1.0 micrograms per gram, there was a positive relation between erythrocyte transketolase activity and dietary thiamine content. Similarly in another series there was a positive relation between the serum transaminase activity and the pyridoxine content of otherwise adequate diets. (Supported by the U.S.P.H.S. and Williams-Waterman Fund.)

2 *Biochemistry*

EICOSATRIENOATE IN THE ERYTHROCYTES OF MONKEYS DEPRIVED OF ESSENTIAL FATTY ACIDS. L.D.Greenberg and H.D.Moon.* Dept. of Pathology, Univ. Calif. Medical Center, San Francisco, Calif.

The fatty acid "spectra" of the lipids of the plasma and erythrocytes (RBC) of monkeys subjected to various dietary deficiencies have been investigated by gas chromatography. Fatty acid methyl esters were analyzed at 190-220° on conventional polyester columns by katherometer with He as carrier gas. An uncharacterized peak representing a small percentage of fatty acid was observed to be present in RBC and more frequently in plasma of control animals. This peak became more prominent in the RBC-fatty acids of monkeys deprived of EFA for a number of months and the relative percent of this fatty acid increased appreciably with increase in the duration of the deficiency. Fractionation of the RBC-lipids on silicic acid and subsequent analysis demonstrated that the fatty acid was limited to the phospholipids. Evidence to support the assumption that the fatty acid is eicosatrienoate (20:3) was obtained by 2methods: 1.Comparison of the retention time of the unknown fatty acid with that of an authentic sample of 20:3 under the same conditions, and 2. by plotting the $\log_{10}$ of the rentention volume according to the method of James (J. Chromatography 2, 552, 1959). The possibility of using RBC-20:3 as an index of EFA deficiency is under investigation. (Supported in part by USPHS Grant A-645-C4 and the Nutrition Foundation, Inc.).

3 *Nutrition*

COMPARISON OF SERUM AND ERYTHROCYTE HEMOLYSATE TRANSAMINASE SYSTEMS. David J. Albert* and Myron Brin. Departments of Biochem. & Medicine, State Univ. of N. Y. Med. Coll., Syracuse.

The alanine transaminase activity of erythrocytes obtained from pyridoxine deficient rats was depressed comparably to the serum enzyme in the same animals. (In weanlings to about 30% normal and in adult rats to 50% of normal in two weeks on treatment.) When pyridoxine was restored to the rats, serum enzyme reverted to normal in three days, whereas erythrocyte enzyme remained depressed. A group of normal rats was treated with carbon tetrachloride orally. After 48 hours, the serum enzyme had risen to 847% of control value, while the erythrocyte value was essentially unchanged. This series of experiments indicated that the serum transaminase and erythrocyte hemolysate transaminase systems are independent enzyme mechanisms in both adult and weanling rats. These data will be presented with a view toward the application of the findings to the evaluation of nutritional status. (Supported by the Williams-Waterman Fund.)

4 *Nutrition*

XANTHURENIC ACIDURIA NOT CORRECTED BY PYRIDOXINE. Sigler, M.H., Sheehy, T.W., Santini, R., and Rubini, M.E. (intr. John B. Youmans.) U.S.A. Tropical Research Medical Laboratory, and University of Puerto Rico School of Medicine, San Juan, Puerto Rico.

Urine xanthurenic acid, a tryptophane metabolite, may reflect body stores of pyridoxine (B_6). In pyridoxine deficient man tryptophane loading results in increased xanthurenic aciduria; this is prevented by the prior administration of pyridoxine. Xanthurenic aciduria was observed in tropical sprue, a disease characterized by malabsorption and an apparent deficiency of folic acid. Five cases of tropical sprue, followed for two to ten days, excreted an average of 15 mgm/day of xanthurenic acid; healthy controls excreted <6 mg/day. This xanthurenic aciduria was increased by tryptophane loading, despite prior administration of pyridoxine (50 mg. i.v.). Although pyridoxine corrects certain macrocytic tropical anemias, such therapy was ineffective in sprue. However, one patient failed to respond normally to folic acid and cyancobalamin (B_{12}) until pyridoxine was given. The xanthurenic aciduria observed in tropical sprue could not be ascribed to an occult pyridoxine deficiency. Furthermore patients with malabsorption due to subtotal gastrectomy or pancreatitis did not excrete excess xanthurenic acid. A possible relationship of folic acid deficiency to tryptophane degradation will be discussed.

5 *Nutrition*

GROWTH RESPONSE & PLASMA GOT LEVELS IN RATS WITH CHRONIC & INTERMITTENT B6 DEFICIENCIES. Samuel D.J. Yeh*, Elba Duran* and Bacon F. Chow. Johns Hopkins Univ., Balto., Md.

Homeostatic mechanism may prevent profound physiol. alterations of the body under many suboptimal conditions. Nutritional requirements may be altered as the result of this readjustment. With growth rate and plasma GOT levels as measures, we wished to follow the different body response to chronic B6 def. & repeated depl. & repl. of this vit. over 56 wks. Male weanling rats from our stock colony were placed on casein sucrose diet suppl. with adeq. supply of all known nutrients except B6. They were div. into 4 groups & pairly fed on the following treatments: A-def.; B-B6 treated; C-def. 6 wks., treated 6 wks. intermit. by oral route; D-repl. by I.P. Our results showed a very close relationship of plasma GOT levels & B6 supplementation. Plasma GOT of group A decr. steadily from a starting level of 87 u.(44 controls) to 13 u. at the end of 30 wks. Group B varied from 70 to 107 u. during the entire period. Rats in groups C & D showed decr. or incr. in accordance with depl. or repl. of B6, but their levels during depl. were never lower than group A. Better growth response was also observed when B6 was supplied. It is concluded that intermit. def. rats may have greater capacity to stand the 2nd depl. & requirement of B6 may be altered due to this homeostatic mechanism.

6 *Nutrition*

CRICKET NUTRITION: UNIDENTIFIED GROWTH FACTOR.
P. Neville*and T.D. Luckey, Dept. of Biochemistry, University of Missouri, School of Medicine, Columbia, Missouri.

Crickets, Acheta domestica L., fed a semisynthetic diet consisting of 30% casein, 42% carbohydrate, 12% cellulose, corn oil, vitamins, salts and cholesterol were found to grow more rapidly when the diet was supplemented with crude material such as yeast, grass or a liver powder. Grass and the liver powder were eliminated as growth stimulators by increasing the cholesterol content of the diet from 0.2% to 0.4%. The best sources of this growth factor(s) were a hog kidney powder, Torula yeast, and dried beef kidney as shown by a significant weight increase over the control in a two week assay.

Some materials tested but found ineffective were the ash of rye and yeast, carnitine, purines and pyrimidines and their derivatives, some sugars, higher levels of B vitamins and some amino acids.

The results of this investigation show that l-arginine can substitute for the growth factor as 1.2% arginine added to the diet produced growth equivalent to 4% yeast or kidney powder. Citrulline but not ornithine would also stimulate growth.

206. NUTRITION–Diet

Biochemistry

THE ANTICARIOGENICITY OF SEED HULLS IN THE COTTON RAT DIET. Kenneth O. Madsen (intr. by E. Beerstecher Jr.) Univ. of Texas Dental Branch, Houston, Texas.

In preliminary studies it was established that there was anticariogenic activity in the hulls of rice, peanuts, cottonseeds and barley. These data were obtained by feeding the finely ground hulls to cotton rats as 25% of the cariogenic diet. In the present study the hulls of rice, peanuts, cottonseeds and pecans were found to be anticariogenic when fed as only 15% of the diet. The anticariogenicity of different varieties of rice hulls was found to be variable. Two varieties contained potent activity, while a third variety contained only slight activity. Rice hulls were fed at various time intervals during the 98-day experimental period. The anticariogenicity of rice hulls was greatest when fed during the first 10-day period after weaning. Caries was arrested, however, when the rice hulls were fed as late as 55 days in the experimental period. The anticariogenicity of dietary hulls was measured after the removal of the submandibular-sublingual salivary glands. These glands were not found to play a role in the rice hull anticariogenic effect, since their removal did not alter the caries scores obtained in the rice hull diet. This study did indicate that these glands may play a role in determining the caries-susceptibility of newly erupted teeth in the cotton rat.

207. NUTRITION–Fats

Physiology

EFFECT OF HIGH FAT DIET ON BLOOD SUGAR, GLUCOSE TOLERANCE, AND WEIGHT OF THE MALE RAT. Enid A. Neidle, Dorothy Fetter, and Hsou-Kao Liu.* N.Y.U. College of Dentistry, New York.

60 male weanling Wistar rats were divided into 4 groups of 15 each. Groups A and C received a low fat diet in which 70% of the calories were derived from carbohydrate. Groups B and D were fed a high fat diet in which 70% of the calories were derived from fat. Rats of Groups C and D were castrated when 4 wk. old. The experiment continued for 16 wk. Blood sugars and body weights were determined weekly. For this period the blood sugars of the rats in Group B were slightly but significantly higher than those of Group A (4 mg%). The blood sugars of Group D averaged 9 mg% lower than those of Group C during the first 8 wk. but in the remaining 8 wk. the blood sugars of Group D were 7 mg% higher than those of Group C, reflecting a marked increase in the blood sugars of the high fat-fed castrates. Glucose tolerance tests done after 12 wk. showed a greater rise in blood glucose in both high fat-fed groups than in low fat-fed animals, but the slopes of the curves were similar. Weight records showed no significant difference until the 10th wk. After that there was a progressive increase in the differences in weight between the high fat-fed and low fat-fed animals, both intact and castrate. The ultimate effect of the high fat diet is to increase slightly the blood sugar, decrease the glucose tolerance, and increase the weight of both intact and castrated male rats.

2 *Physiology*

THE INFLUENCE OF A HYPERCALORIC DIET ON GROSS BODY AND ADIPOSE TISSUE COMPOSITION IN THE RAT. Cecil Entenman and S. C. Peckham*, U. S. Naval Radiological Defense Lab., San Francisco 24, Calif.

To ascertain the alterations in gross body composition and in number and composition of adipose tissue cells during development of obesity, weanling, weight-matched rats were fed hypercaloric diets of Purina laboratory chow. The hypercaloric diet fed was similar to diet No. 10020 used by Michelsen (J. Nutr. 57, 541, 1955). After intervals of ten and thirty-four weeks on these diets, representatives of both groups were sacrificed, the epididymal depots excised and analyzed for fat-free dried residues, and DNA. Results consistently show that the DNA content of this adipose tissue, and hence the number of cells, is a function of the amount of fat deposited. Carcass analyses not only showed that the generally heavier rats fed the hypercaloric diet had a greater absolute amount of fat but also that this fat was superimposed on a lesser absolute protein-water-ash mass than that of the weight-matched controls on the standard pellet diet, regardless of the weight relationships at the time of sacrifice. Both the fat-free mass and water content decreased. The implication of these results to the nutritional evaluation of diets will be presented.

3 *Physiology*

IN VITRO METABOLISM OF SHORT-CHAIN FATTY ACIDS IN BOVINE RUMEN EPITHELIAL TISSUE. K. Knox, E. E. Flamboe, R. M. Johnson, and G. W. Ward (intr. by G. Filley.) Colo. State Univ., Fort Collins, Colo.

The metabolism and interaction of short-chain fatty acids in sheep rumen epithelial tissue has been reported by a group of workers in England. This has provided impetus for the present study in which similar metabolic measurements were made using rumen epithelial tissue from the immature and the mature bovine. The Q_{O2} value obtained from the Warburg micro-respirometer in which rumen epithelium had been incubated in either glucose, acetate, propionate, or butyrate or a combination of these was used as a criterion for determining metabolism and interaction of short-chain fatty acids. The results of this study indicate: 1. All four substrates used were metabolized by the immature and the mature bovine rumen epithelium. 2. In close agreement with results obtained from sheep, butyrate served as the best substrate in both age groups. 3. Higher Q_{O2} values were observed in both age groups where a mixture of fatty acids was used as a substrate. 4. In the immature the utilization either of butyrate, glucose, or acetate as single substrates was greater and more persistent when compared with their utilization in the mature bovine rumen epithelium.

4 *Biochemistry*

STUDIES ON A FACTOR CAUSING PERICARDIAL EDEMA IN CHICKS AND ITS OCCURRENCE IN SOME OLEIC ACIDS. Stanley R. Ames, William J. Swanson* and Philip L. Harris. Research Laboratories, Distillation Products Industries, Division of Eastman Kodak Company, Rochester, New York.

Continuing studies on a factor causing pericardial edema (PE) (also termed hydropericardium) in chicks have led to the development of a rapid and sensitive bioassay. The diet used is a modification of that described by Brew, et al., J. Assoc. Offic. Agr. Chemists 42, 120 (1959), and consists of purified casein, gelatin, cerelose and 16% test fat. Day-old chicks are fed the test diet immediately, since prefeeding of stock diet lowered incidence of PE. Sub-groups sacrificed at 10 and 14 days showed a higher incidence of PE than at 21 days. Using this procedure, some samples of oleic acid were shown to contain the PE-producing factor. Glycerol esters prepared from the oleic acids containing this factor also produced PE. Molecular distillation of oleic acid concentrated the PE-factor in the residue fraction and, of glycerol esters, in the first 10% distillate. Bioassay results on other fats and fatty acids will be presented.

5 *Pharmacology*

SPECIFIC POISONOUS EFFECT OF HYDROCARBONS. Phil Truan,* G. E. Price* and R. H. Beutner. College of Osteopathic Medicine and Surgery, Des Moines, Iowa.

Our previous work has shown that saturated fatty acids at a high concentration (50%) in the diet will kill rats within a week to ten days. (See article in this issue of Federation Proceedings.) When rats were fed in the manner previously described on a diet containing 50% of a liquid hydrocarbon, they developed a peculiar symptom of poisoning, never previously described. A great deal or possibly all of the ingested mineral oil was excreted through the skin. The rats looked as though they had been bathed in the oil. The fur of the rats was soaked in the oil. Apart from the mineral oil, the rats received the previously described diet consisting of casein, glucose, salt mixture and vitamin mixture as well as water ad lib. (Supported by grants from the National Heart Institute, Bethesda, Md. and from the American Heart Association of New York.)

6 *Pharmacology*

TOXICITY OF A UREA FILTRATE FRACTION FROM A FATTY FEED BY-PRODUCT. J.I.Munn*, S.A.Koch* and O.G.Fitzhugh. Div.of Pharmacology, Food & Drug Admin., U.S.Dept.of Health, Education, and Welfare, Washington, D.C.

During the fall of 1957 a heavy incidence of an edema condition in broiler-type chicks appeared which was related to the unsaponifiable portion of the fat in the feed. Later the urea filtrate fatty acid fraction was shown to cause fatalities in young rats. In order to study this problem further the non-adducting fatty acids prepared from the fatty-by products after saponfication and urea fractionation were fed to 5 groups (12 per group) of weanling rats for 12 weeks at the 5, 2, 1, 0.5 and 0.25% levels. The stock diet and the urea adducting fatty acids served as controls and were fed to equal sized groups of animals. It was found that in both male and female rats there was a slight depression of growth and a decreased food consumption at the highest level. Gross pathology was negative. The organ weights as related to the total body weights were increased at the 5% level. Histopathology was negative except for a slight increase in the size of the liver cells at the 5% level. Blood indices were normal except for a slight decrease in the hematocrit at the highest level. Blood chemistries consisting of electrolytes, sugars, cholesterol and phospholipids were also normal except for alkaline phosphatase which was increased about 4 fold over the controls at the 5% level. This finding is suggestive of early liver dysfunction.

7 *Nutrition*

EFFECT OF NUTRIENT DENSITY AND RESTRICTED NUTRIENT INTAKE ON DIET UTILIZATION BY THE LAYING HEN. Sherman P. Touchburn and Edward C. Naber (Intr. by W. E. Krauss), Dept. of Poultry Science, Ohio Agric. Expt. Station, Columbus.

Previous studies have shown that a high rate of egg production can be maintained by hens fed rations varying widely in nutrient density. The term nutrient density is used to describe changes in the dietary concentration of individual nutrients under conditions where they maintain a constant relationship to each other. Recent experiments have shown that feed utilization for egg production improved as the nutrient density of the diet increased. When five diets of different nutrient density (12 to 20 per cent protein; 720 to 1200 Calories of productive energy per pound) were fed to laying hens, feed utilization efficiency did not parallel the changes in nutrient density of the diet. The effect was most apparent in the diet of highest density where the relative efficiency was poorer then expected. An increasing proportion of the energy in the higher density diets was provided by animal fat. Attempts to improve utilization of the highest density diet by supplementation with lipases, bile salts and cholagogues failed. Limiting consumption of the highly concentrated diet showed that nutrient utilization was improved by preventing overconsumption. Reasons for overconsumption of the high density diets may have been: (1) that since most of the calories were supplied by fat, blood glucose level was not raised sufficiently to satisfy appetite, (2) the highly concentrated nature of the diet did not provide adequate physical stimulus in the digestive tract to satisfy appetite.

8 *Nutrition*

ACETOACETATE AND β-HYDROXY BUTYRATE UTILIZATION IN SHEEP. W. H. Pfander and R. L. Reid*, Univ. of Mo. and CSIRO Sheep Biology Laboratory.

Data presented previously (J. An. Sci. 18:1560) suggested that ketone removal was a rate limiting step in acetate utilization measured by tolerance tests. Acetate half life (y) in sheep fasted three days was related to initial ketone level (x) ($y=22\pm8.25x$). We have explored these relationships in further tolerance tests on ten sheep injected with sodium salts of acetoacetic (AA) and beta hydroxy butyric (BHB) acids. The (AA) half life (t/2), in minutes, was 8.4 ± 1.6 and was unaffected by age or nutritive status. The t/2 of BHB formed from AA in vivo by sheep on the indicated food intake was: maintenance, 39.7 ± 15.3; 1.5 maintenance, 33 ± 4.9; ad libitum, 15.5 ± 5.4. Similar, but less variable, t/2's were obtained for injected BHB at maintenance and 1½ maintenance. Four -8% of injected BHB was converted to AA. The rate limiting step in acetate utilization must be beyond the conversion of AA to BHB.

XUM

9 *Nutrition*

INTAKE AND FECAL EXCRETION OF FAT BY 36 PREADOLESCENT GIRLS. Larue B. Stier, Doris D. Taylor, Patricia A. Goodman, and June K. Pace (intr. by Callie Mae Coons). Human Nutrition Research Division, USDA, Washington, D. C.

In a Southern Region cooperative study to determine metabolic patterns in preadolescents, data were obtained for 36 healthy girls 7 to 9 years of age. Metabolic materials were collected during three controlled diet series in 1954, 1956, and 1958 for 64, 56, and 48 days, respectively. The diets consisted mostly of a typical assortment of ordinary foods, with level of protein intake as the chief variable. For all subjects and series, the average daily intake of fat ranged from 66 to 79 grams and provided from 30 to 32 percent of the total calories. The fat intake per kilogram of body weight ranged from 2.2 to 2.8 grams per day. Average daily excretion of fecal fat ranged from 2.6 to 3.6 grams total or about 0.1 gram per kilogram of body weight. Apparent digestibility of the fat ranged from 94 to 96 percent. The amount of vegetable and animal fats in the diets, as well as the fatty acid content of the diets, will be reported. Relationships between fat intake and fecal fat, calcium, and magnesium, as well as nitrogen retention, will be discussed.

10 *Nutrition*

SWELLING OF RAT LIVER MITOCHONDRIA IN ESSENTIAL FATTY ACID DEFICIENCY. T. Hayashida* and O. W. Portman. Harvard School of Public Health, Boston, Mass.

Various studies have suggested that essential fatty acids (EFA) may be important in the biochemical properties and structural integrity of the mitochondrial membrane. Studies of the effect of the nature of the suspending medium on the swelling of liver mitochondria from normal and EFA deficient rats were made. Higher swelling rates of EFA deficient mitochondria were observed using 0.30 M sucrose medium. Respiratory inhibitors (cyanide, Na amytal, and antimycin A) inhibited the swelling of both control and EFA deficient mitochondria. ATP prevented the swelling of control but not that of EFA deficient mitochondria. EDTA showed a protective action for both groups of mitochondria. Serum albumin inhibited the swelling of control but not that of EFA deficient mitochondria. Cholate accentuated the swelling patterns of both groups of mitochondria. Fatty acid analyses for control and EFA deficient mitochondria will be presented. These studies support the suggestion that EFA play a role in the structural stability and function of the mitochondria. (Work performed during Dr. Portman's tenure as an Established Investigator of the American Heart Association.)

11 *Nutrition*

METABOLISM OF SATURATED FATTY ACIDS BY THE RAT. S. L. Kirschner* and Robert S. Harris, Department of Food Technology, Mass. Inst. of Tech., Cambridge.

Four typical saturated fatty acids (C_4, C_8, C_{12}, C_{16}) were labeled with C^{14} on the carboxyl carbon. Each was blended with identical mixtures of food fatty acids, esterified, and then mixed with safflower oil (1:1). Young adult male rats (152-174 gm) received 0.8 ml of these labeled oils by stomach tube and were placed in glass (Delmar) metabolism units. Respired CO_2, and urine and feces, were collected during 48 hr. The rats which received the fats containing labeled butyric, caprylic, lauric and palmitic acids, respectively, respired 60%, 55%, 40% and 25% of the radioactivity during 8 hr., and 79%, 76%, 59% and 49% during 48 hr. The radioactivity excreted in the feces during 48 hr. was 0.5%, 0.5%, 8.1% and 12.2%, respectively. Therefore, the radioactivity of these saturated fatty acids was absorbed from the gastro-intestinal tract and respired in the breath at rates inversely proportional to the chain length.

12 *Nutrition*

THE EFFECT OF DIETARY FAT ON METABOLIC PATTERNS IN RATS ON SEVERELY RESTRICTED DIETS. L. Arnrich, J. L. Dupont* and J. E. Clifford*. Food and Nutrition, Iowa State Univ., Ames, Iowa.

It has been shown that fat, substituted isocalorically for dextrin in diets of adult rats deprived of protein and restricted in energy allowance, may reduce tissue catabolism. This investigation has been extended to study patterns of liver phosphatide metabolism with P^{32}. The effects of hormones known to modify protein metabolism have been explored also. As dietary stress increased total liver lipid P, total liver phosphatide activity, and relative amounts of choline-containing liver phosphatides decreased progressively on the low fat regime. With isocaloric substitution of 20% of the carbohydrate by fat, a change in these constitutents with increasing stress was seen only when the most severe restriction was imposed. Testosterone injections augmented losses of body nitrogen observed in rats on low-fat low-nitrogen diets and restricted in calories, though the effect of this hormone was expected to be anabolic. The higher nitrogen losses were accompanied by increased weight losses, increased adrenal weights, decreased adrenal ascorbic acid concentrations, and greater mortality rates. The inclusion of fat into the diets protected rats from the detrimental effects of testosterone. Changes produced by cortisone and ACTH injections were not significant.

13 *Nutrition*

NUTRITIVE VALUE OF HEATED FATS. Leo Friedman, Wm. Horwitz*, Glen M. Shue*, and David Firestone*. Food & Drug Administration, Washington, D. C.

Alteration in nutritive value of fats subjected to continuous heating over long periods of time has been under study in this laboratory. Growth response as well as tissue and organ changes in rats have been used to measure such alteration. Cottonseed oil, heated continuously for 7 to 8 days at 225° C, was fed to rats at levels of 10 and 20% of a sucrose-casein type diet for a period of 6 months. Comparisons were made with animals fed unheated oil under the same conditions. Decreased food efficiency, increased liver weight, and, in the females, enlarged thymus and pituitaries, were observed in the heated oil groups. Short-term restricted-feeding studies of the type described by E. E. Rice et al (J. Nutr. 61:253, 1957) were used to guide separation from the heated oil of a fraction that is growth-inhibiting at levels of 2.0% in the diet and which at 0.5 to 1.0% causes increased liver weights. This fraction prepared by molecular distillation and urea fractionation, consisted primarily of monomeric material that does not adduct with urea.

208. NUTRITION–Growth

1 *Physiology*

CALORIGENIC RESPONSE TO HIGH CARBOHYDRATE INTAKE IN WEANLING RATS. F.W. Heggeness (intr. by E.S.Nasset.) Univ. Rochester Sch. Med. & Dentistry, Rochester, N.Y.

Weanling male albino rats of the Holtzman strain housed in a constant temperature room were fed ad libitum a 60% glucose, starch, or galactose diet. Daily resting but not fasting oxygen consumption (D.T. Watts and D.R.H. Gourley. Proc. Soc. Expt. Biol. and Med., 84:585,1953) was determined. Metabolic rate was calculated as Cals/Kg $^{3/4}$/hr., assuming a respiratory quotient of 0.80. Prior to the start of feeding, metabolic rate was 4.2 Cals/Kg $^{3/4}$/hr. Twelve hours after feeding of glucose diet it had increased to 6.0 Cals. Rate continued to rise until the fourth day, after which it gradually decreased, approaching 5.0 Cals/Kg $^{3/4}$/hr. by the ninth day. The calorigenic response to a 60% starch diet was delayed 24 hours and did not persist as long. Rats fed the galactose diet showed failure of growth and only a small rise in metabolic rate. Fasted 100 gm. animals of the same strain show no calorigenic response to realimentation.

2 *Biochemistry*

A HEAT-INACTIVATED FACTOR REQUIRED FOR ASEXUAL GROWTH OF HYDRA. David L. Claybrook* and Robert E. Eakin. Clayton Fndn. Biochemical Inst. and Dept. of Chemistry, Univ. of Texas, Austin.

In order to study the nutrients required for asexual growth in hydra, a quantitative assay system has been developed to determine the effectiveness of various substances in stimulating budding. A micro-injection technique for feeding individual hydras has been devised whereby measured quantities of test material, either soluble or insoluble, may be introduced into the coelentron. It has been found that heating the brine shrimp Artemia (on which the hydra are maintained) progressively destroys their capacity to support asexual growth. Artemia heated seven minutes at 70°C. are adequate to maintain hydra, but the budding rate is greatly reduced. The heat-inactivated factor(s) in Artemia can be replaced by some other natural materials, with yeast and liver being the most active sources. Such substances must be fed since any addendum to an adequate inorganic aqueous environment does not stimulate budding. A variety of known vitamins, amino acids, and other nutrilites have been fed with uniformly negative results. Properties of the substances replacing the heat-inactivated nutrient will be discussed.

3 *Pharmacology*

THE EFFECT OF UREASE INJECTIONS ON GROWTH OF RATS. W. J. Visek and Hung-Chen Dang (intr. by L. J. Roth.) Dept. of Pharmacology, University of Chicago.

Twenty weanling Sprague-Dawley male rats weighing 40-50 g were injected over a four-week period with a total of 30 units of twice recrystallized jackbean urease in doses starting at 0.5 units per injection and progressing to 5 units per injection. Control animals received equivalent volumes of physiological saline. The diet fed ad libitum contained solvent extracted soybean meal (50% protein) 31.4; cellulose 2.2; vegetable oil 8; U.S.P. XIV salt mixture 4; vitamins in sucrose 2.2; and sucrose 52.2. No differences in growth were noted during the injection period. However, in the 28-day period following cessation of injections, the experimental rats gained 107.8 g and required 4.64 g of feed per g of gain. Corresponding values for the 10 control animals were 94.3 and 5.39. The anti-sera produced by the experimental animals protected normal animals against lethal doses of the antigen. Previous work by us has shown suppression of C-14 urea hydrolysis by trace dietary levels of three antimicrobial agents. Our present findings indicate that possession of specific antibodies to an urea splitting enzyme makes possible more efficient growth in rats.

4 *Pathology*

FURTHER STUDIES ON KIDNEY GROWTH: COMPENSATORY RENAL GROWTH FOLLOWING UNILATERAL NEPHRECTOMY IN THE RAT. D. S. Miyada and N. B. Kurnick (intr. by D. T. Imagawa) Univ. of Calif. School of Med., Los Angeles and Veterans Administration Hosp., Long Beach, California.

Quantitative evidence is presented for the occurrence of both hypertrophy and hyperplasia in kidney during compensatory renal growth following unilateral nephrectomy. The first few days after unilateral nephrectomy of rats, there was a rapid increase in kidney mass amounting to approximately 30% at 4 days with essentially no change in the DNA content per kidney. Subsequently, between the 6th and 16th days post-nephrectomy, there was a 15% rise in kidney mass corresponding to an increase of 30% in the DNA content per kidney. The rapid increase in kidney mass in the first few days after unilateral nephrectomy with very little change in the DNA content per kidney reflects the role of hypertrophy while the subsequent increase in mass, now less rapid, with a significant increase in the DNA content per kidney reflects the role of hyperplasia. Compensatory renal growth was followed by determinations of mass, RNA, DNA, N, P, and rate of incorporation of glycine-2-C14 over a period of 16 days, by which time it had about run its course.

(Supported by grants from the United States Public Health Service and The American Cancer Society.)

209. NUTRITION—Proteins

1 *Pathology*

THE SERUM GLYCOPROTEIN RESPONSE IN TUMOR-BEARING RATS ON A PROTEIN-FREE DIET. Henry E. Weimer and Hisako Nishihara*. Department of Infectious Diseases, University of California Medical Center, Los Angeles.

Two groups of adult, male Sprague-Dawley rats were fed a protein-free diet until weight losses of 11 and 25% were attained. They were then inoculated subcutaneously with a suspension of the Walker 256 carcinosarcoma and continued on the diet. The rats were sacrificed after 14 days. The tumors were weighed and the protein-bound carbohydrates and proteins of the seromucoid fraction and of whole serum as well as the hemoglobin and hematocrit values of blood were determined. Analyses were also made on non-inoculated controls fed the protein-free diet and sacrificed after weight losses of 11, 25, 33 and 40% and on tumor-bearing rats maintained on a stock diet. The components of the seromucoid fraction were little affected by protein depletion but were significantly increased in tumor-bearing rats irrespective of diet. The concentration of non-seromucoid glycoprotein was significantly reduced in both protein depleted and tumor-bearing rats. The conclusion was drawn that the response of the seromucoid fraction to tumor growth is not impaired by protein depletion.

2 *Nutrition*

EFFECT OF VARIOUS CARBOHYDRATES ON UTILIZATION OF LYSINE BY RATS. Yet-Oy Chang and Helen J. Souders. (introd. by C. A. Storvick). Div. of Home Econ., Univ. of Wyo., Laramie.

Weanling male rats were divided into five groups, receiving the following carbohydrates: potato starch, corn starch, sucrose, dextrin and glucose, with wheat gluten as the source of protein. Growth and food consumption were recorded. Nitrogen balances and lysine utilization during growth as well as during and after protein depletion were studied. Lysine concentration in blood plasma and livers of the rats were determined. Rats fed sucrose were the slowest growing, while the dextrin-fed rats were fastest growing. There were no significant differences in nitrogen balance among the five groups. Lysine utilization was significantly lower in the potato starch group in all periods. The glucose group showed the greatest utilization during protein repletion. The other three groups were similar in their lysine utilization. Free lysine concentration of blood plasma from rats fed glucose was somewhat higher, whereas that from rats fed potato starch was somewhat lower compared to the other three groups. The free and total lysine concentration in the livers of the dextrin-fed rats was higher than the other four groups.

3 *Nutrition*

ENERGY OUTPUT OF PATIENTS FOLLOWING SURGERY. Stanley Levey, Nancy Czarnecki,* Harvey Krieger* and William E. Abbott.* Dept. of Surgery, College of Medicine, Western Reserve University, Cleveland, Ohio

In seeking why postoperative patients require more calories and possibly more nitrogen to attain nitrogen balance than they need during the preoperative period, studies were undertaken to determine the energy output of such patients. Measurements of the daily energy production using indirect calorimetry were carried out on four individuals undergoing a hernia repair and on one patient after a partial gastric resection. For comparison purposes the preoperative period was matched day by day to simulate what the patient would receive postoperative in the form of nutrition, the manner it was given and also the degree of ambulation. All the patients had uneventful postoperative courses and showed little elevation in body temperature. No significant increase in energy output was noted during the operation or the postoperative period in this study. Also no difference in the amounts of fat, protein and carbohydrate oxidized could be related to the surgery. This suggests that the energy expenditure of the postoperative patient need not be different from that of the preoperative state. This work was supported in part by a U.S. Army contract (DA-49-007-MD 922) and the John A. Hartford Foundation.

4 *Nutrition*

THE INFLUENCE OF QUALITY OF DIETARY PROTEIN ON THE SUSCEPTIBILITY OF RATS TO INFECTION WITH TRYPANOSOMA CRUZI. Robert G. Yaeger* and O. Neal Miller, Tulane Univ. Sch. of Med., New Orleans, La.

The prevalence of malnutrition where parasitic diseases are most common prompted a study of the relationship between quality of protein in the diet and susceptibility to Chagas' disease. 5 isonitrogenous diets (19.6% protein) were made: casein (A, control), gluten (B), gluten + lysine (C), gluten + lysine + methionine (D), gluten - methionine (E); the remainder of each diet was the same. 20 male rats were used per group, fed ad lib. After 3 weeks, 10 rats from each group were inoculated with T. cruzi. Parasitemia was determined 3 times per week by counting the number of trypanosomes/200 microscopic fields (430x) in cover glass mounts of fresh tail blood. All rats were sacrificed after 6 weeks and tissues taken for study. Susceptibility, based on parasitemia and tissue damage was least in Gp.-A and most severe in Gp.-B. Addition of lysine to the gluten diet (Gp-C) greatly decreased susceptibility but not to the level of Gp.-A. Addition of methionine to the gluten diet (Gp.-E) also resulted in a decrease in susceptibility. When lysine and methionine were added to the gluten diet, susceptibility was decreased, < Gp-B but > Gp.-C, suggesting additional studies on amino acid balance.

5 *Nutrition*

THE USE OF NITROGEN/CREATININE RATIOS IN RANDOM URINE SPECIMENS TO ESTIMATE DIETARY PROTEIN. R. C. Powell, E. M. Baker II, and I. C. Plough. (intr. by John B. Youmans). U.S.Army Medical Research and Nutrition Laboratory, Denver, Colorado

Evaluating the adequacy of dietary protein is one of the important problems confronting nutritional survey teams. Because of the large population sampled, it is necessary that any test for this purpose be easily and rapidly performed. Many studies have shown that 24 hour urine total nitrogen values vary with dietary protein levels. To circumvent the difficulties involved in collecting a timed urine specimen, it has been suggested that urinary nitrogen be expressed per gram of creatinine. Takagi and Masuda, Repts. Inst. Sci. Labour 49:13, 1956, have reported such a survey correlating dietary protein and urinary nitrogen expressed in terms of creatinine. To further clarify this relationship the following study was undertaken. Urine nitrogen and creatinine determinations were made on timed urine samples in six normal human volunteers, while dietary protein was varied from 75 to 125 gms per day. The diurnal variations of urinary nitrogen and creatinine were found to be similar. The confidence limits for estimating dietary protein from the urinary nitrogen expressed per gram of creatinine will be presented.

6 *Nutrition*

"RESERVE PROTEIN" -- a misnomer? E. Halac, Jr. (introduced by L. E. Holt, Jr.) Department of Pediatrics, New York University College of Medicine, New York.

It is well known that nitrogen retention can be increased by increasing nitrogen intake. The term reserve protein has been applied to the increment of nitrogen in the body. The implications of this term are two: (1) that the nitrogen is actually in the form of protein and (2) that the increment of retained nitrogen actually functions as a store which is useful in time of need. These two concepts do not stand critical analysis. It can be shown that a substantial fraction of the nitrogen increment consists of non protein nitrogen in the cells and the extracellular fluids. An experiment was devised to determine whether a high protein diet would protect the rat from subsequent protein deprivation. The high protein diet failed to prolong the life of animals subsequently deprived of protein. Thus the additional nitrogen content of their bodies at the start of the experiment did not function as a store. It would seem preferable to use the term "labile nitrogen" rather than "reserve protein".

7 *Nutrition*

THE UTILIZATION OF PROTEIN BY COLLEGE WOMEN ON A TEN GRAM PROTEIN DIET BEFORE AND DURING STEPWISE SUPPLEMENTATION WITH BEEF OR HADDOCK. Pela F. Braucher (intr. by Georgian Adams.) College of Home Economics, University of Maryland, College Park, Md. (Contribution from Northeast Regional Project, NE-37)

There is evidence that the human body can adjust to constant low protein intake and attain nitrogen equilibrium. Two experiments similar in design were carried out to study the metabolic response to various low protein intakes. Nineteen college women ate a diet containing 10 grams of protein for 12-15 consecutive days. This daily diet was then supplemented stepwise with 10, 20, 25, 30, 35, and 40 grams of beef protein for half the subjects and haddock protein for the other subjects. Each level of protein supplement was eaten for 6-9 days. The total period of experimentation was 42-51 days. Body weight was controlled by adjustment in the caloric intake of each subject during the experiments. On the 10 gram protein intake, nitrogen retention became positive or only slightly negative after 9 days. With supplementation, nitrogen retentions increased gradually to a peak at 33-39 days (total protein intake of 35 grams), then decreased somewhat regardless of protein intake. The positive nitrogen retention of the 3-day metabolic periods showed a rhythm similar to that of a former experiment in which the protein intake of 44 grams per day was constant.

8 *Nutrition*

STUDIES ON THE NUTRITIONAL VALUE OF DEFATTED FISH FLOUR. A.B. Morrison and J.A. Campbell. Food and Drug Lab., Dep't Nat'l Health and Welfare, Ottawa, Canada.

Protein efficiency ratio (P.E.R.) values found for 7% and 10% fish flour were higher than those for comparable levels of casein, but similar values were found for both proteins at the 15% level. Fish flour was added to whole wheat flour or to white bread prior to baking, and the mixtures were fed to growing male rats at the 10% protein level. Addition of 2, 4, or 6 lb. of fish flour per 100 lb. of wheat flour increased the P.E.R., in a stepwise manner, from 1.49 to 2.80, but had no effect on organ weights or liver lipid levels. Addition of 10% fish flour to white bread made with or without 4.2% milk solids increased P.E.R. values by 82% and 198% respectively, and significantly reduced liver lipid levels. P.E.R. values for bread and flour diets were found to be a direct function of the lysine content of the diet.

9 *Nutrition*

THE BIOLOGICAL EVALUATION OF PROTEIN QUALITY. B. L. Oser, S. Carson* and M. Oser* Food and Drug Research Laboratories, Inc., Maspeth, New York.

Evaluations of the protein quality of foods and food ingredients were carried out following the procedure described by Chapman et al., with and without modifications. Bread, grains, cereals, casein, non-fat milk solids and dried egg protein were studied at several feeding levels of protein based either on iso-nitrogenicity or N x a factor. Amino acid supplementation of a series of gluten cereal and bread samples were examined at one or more levels of dietary protein intake. Only protein sources of minor or moderate efficiency were significantly improved by lysine supplementation. The effects of the nutritional status of the rats at the start of the test, as reflected by the quality of the diet fed during lactation and immediately post-weaning, on the responses in these protein tests were examined. Although differences in final 4-week values did not differ significantly, with better early nutrition, some improvement was observed during the initial 2-week period. Comparison of several good and poor quality proteins at graded levels of incorporation revealed differences in slopes. Good proteins are adequately evaluated at 10 per cent dietary protein concentrations. Proteins of lesser quality perform more adequately when evaluated at 15 per cent of the diet.

210. NUTRITION–Trace Elements

1 *Nutrition*

ANOMALOUS DEVELOPMENT OF THE INNER EAR IN OFFSPRING OF MANGANESE-DEFICIENT RATS. Lucille S. Hurley, Eloise Wooten*, Gladys J. Everson and C. Willet Asling*. Univ. of California, Davis and Berkeley.

Recent studies demonstrating delayed development of righting reflexes in offspring of Mn-deficient rats (Hurley and Everson, Proc. Soc. Exp. Biol. Med. 102: 360, '59) directed attention to the vestibular apparatus. Ossification of the inner ear was studied in young of Mn-deficient and -supplemented rats, in cleared specimens stained with alizarin red S. The pattern of ossification appeared to be the same for both normal and deficient young, but deficient offspring showed a pronounced retardation. More than half (56%) of 39 deficient newborn young showed less development of the osseous labyrinth than was seen in any of 22 normal newborn young. Only 15% of deficient newborn young, as compared with 73% of normal newborn young, showed development beyond the minimum observed in normal newborns. Measurements of various portions of the otic capsule in young from 6 to 18 days of age revealed a retardation of development in Mn-deficient young of about 2 days, and a reduction in size of the vestibular component which appeared to be independent of body size. The results suggest that an abnormality of the inner ear may be a causative factor in the congenital ataxia produced by maternal Mn deficiency. (Supported by grant A-1340 from U. S. P. H. S.)

2 *Nutrition*

FURTHER STUDIES ON NUTRITIVE EFFECTS OF SELENIUM FOR CHICKS. M. C. Nesheim* and M. L. Scott, Cornell Univ., Ithaca, N. Y.

Selenium has been shown to replace some of the nutritive effects of vitamin E when included in certain diets for chicks. Also when diets containing Torula yeast are fed to chicks, selenium is required for maximum growth even when vitamin E is supplied in adequate amounts. (Nesheim and Scott, J. Nutrition 65: 601, 1958.) To further study the nutritive effects of selenium, attempts were made to formulate experimental selenium-deficient diets not containing Torula yeast. Since the protein constituents of the purified diet ingredients were found to contribute the major amounts of selenium to these diets, appropriate modifications were made wherein part of the nitrogen was supplied by isolated soybean protein and part by crystalline amino acids, or completely by crystalline amino acids. Chicks receiving these diets readily developed exudative diathesis, but showed no growth improvement from added selenium above that obtained with added vitamin E. The selenium content of the basal diets used was determined by activation analyses to be 0.04 to 0.07 mg per kg of diet. Either selenium or vitamin E supplementation completely prevented exudative diathesis in these experiments. Thus the selenium requirement of the chick for growth appears to be less than 0.04 mg per kg of diet.

3 *Nutrition*

HEMATOLOGICAL RESPONSES TO COPPER AND IRON DEFICIENCY IN CHICKENS. C. H. Hill and G. Matrone. N. C. State College, Raleigh, North Carolina.

The hematological response to iron deficiency has been uniformly reported as being hypochromic and microcytic. The response to copper deficiency has been reported as hypochromic and microcytic by some, while others report a normocytic normochromic response. In order to determine the response in the fowl, chicks were fed a dried skim milk-cerelose basal supplemented with Wesson Oil, vitamins, manganese, methionine, arginine, and glycine. Iron, as the sulfate, was added at 0, 40, and 80 ppm. Copper, as the sulfate, was added at 0, 4, and 8 ppm. All combinations of the copper and iron supplements were fed. By the 19th day, both the copper and the iron deficient birds were anemic. The anemia in the iron deficient birds was hypochromic; microcytosis was not pronounced. The anemia in the copper deficient birds was normochromic, normocytic. The difference in hemoglobin content in these chicks was accounted for entirely by the difference in erythrocyte numbers. Iron deficiency had no effect on erythrocyte numbers during this period. (Supported by a grant from the Frasch Foundation.)

211. OBESITY

1 *Physiology*

Physical Activity and Human Obesity. Anna M. Chirico and Albert J. Stunkard (intr. by F. C. Dohan). Dept. of Medicine and Psychiatry, University of Pennsylvania, Philadelphia.

It has been suggested that the pathogenesis of obesity may be dependent in part upon relative physical inactivity. This hypothesis is supported by data obtained from studies comparing physical activity and attitudes toward activity of obese men and women. The present report compares physical activity, measured by pedometers and attitudes toward activity, estimated by questionnaires and sentence completion tests, of 25 obese and 25 matched non-obese men. Results are contrasted to those obtained from 14 matched pairs of obese and non-obese women. Physical inactivity was statistically correlated ($P < 0.01$) with the presence of obesity in these women who walked less than half as far as the non-obese controls. A similar, but less significant trend, was noted in men. The tendency of obese women to respond passively to boredom and despondency and to be sociably inhibited differentiated them from the non-obese women. The reactions of the obese males on the other hand were similar to those of the non-obese group.

2 *Pathology*

ALPHA-GLYCEROPHOSPHATE OXIDATION IN THE TISSUES OF OBESE HYPERGLYCEMIC MICE AND NONOBESE CONTROLS. George H. Fried and William Antopol. Levy Laboratories, Beth Israel Hospital, New York

The unique features of carbohydrate metabolism in mice with the obese hyperglycemic syndrome, particularly the high glycogen turnover in liver, are suggestive of changes in underlying enzymatic mechanisms in these animals. Since alpha-glycerophosphate oxidation links fat and carbohydrate metabolism, a comparison of levels of alpha-glycerophosphate oxidase (AGPO) and alpha-glycerophosphate dehydrogenase (AGPD) in kidney, liver, brain, and muscle (rectus) of obese and normal mice was carried out using 2-(p-iodophenyl)-3-(p-nitrophenyl)-5-phenyl tetrazolium chloride (INT) as redox indicator. Dehydrogenase levels were determined by including phenazine methosulfate and NaCN in the assay procedure. DPN-dependent and non-DPN-dependent pathways were assayed simultaneously. In both groups of mice, DPN-dependent AGPO and AGPD were much higher than the corresponding non-DPN-dependent enzymes in kidney, liver, and muscle; the opposite held true in the case of brain. Levels of AGPO (DPN-dependent) in kidney, brain, and muscle were similar in obese and normal animals, while liver AGPO levels in the obese group were twice those of controls. Similar results were obtained for AGPD. In the absence of DPN the difference in liver enzyme levels in obese and control groups was increased. (Supported by Grant H2670 and H3027(R_1), National Institutes of Health, USPHS)

3 *Nutrition*

SPECIFIC GRAVITY AND TOTAL BODY WATER IN MEN AGED 60 OR OVER. Jeffrey H. Fryer (intr. by L.A. Maynard) Grad. Sch. of Nutrition, Cornell Univ., Ithaca, N.Y.

To permit the determination of specific gravity in older age groups the technique of weighing in water has been adapted so that 15 cms. of the subject's head, measured vertically, remains above the surface. A correction factor can then be applied to give the true specific gravity with a standard error of estimate of .0035, equivalent to less than 2% of body fat. The mean specific gravity of 53 men aged 60 to 89 was 1.0354 which is significantly lower than comparable observations on younger men. The difference may indicate a relative increase in low density tissue, such as fat or water, and/or a relative decrease in high density tissue, such as bone mineral or cell solids. Specific gravity may vary considerably in a given height-weight range. Mean total body water content, as measured by the N-acetyl antipyrine space, was 52.4% of body weight which is also lower than that of younger groups. However the relationship of total body water to specific gravity was relatively inconstant and either factor alone may be misleading. It is concluded that there is an age effect tending to lower body specific gravity and water content, the significance and mechanisms of which remain to be worked out. (Supported by grants from the Nutr. Found. and the Natl. Inst. of Arth. and Met. Dis. P.H.S.)

XUM

4 *Nutrition*

BODY COMPOSITION CHANGES IN MEN DURING WEIGHT LOSS ON A PROTEIN DEFICIENT DIET. J.Manniello, E.Harmuth and J.H.Fryer(intr. by H.H.Williams).Grad.Sch.Nutr.Cornell Univ.,Ithaca,N.Y.

Changes in body composition as determined by specific gravity, N-acetyl antipyrine (NAAP) space, thiocyanate (SCN) space, basal O_2 consumption, creatinine excretion and skinfold measurements were studied in four healthy young men subjected to a diet deficient in protein and calories for 28 days after a control period. Nitrogen balance was studied continuously throughout this period. Mean weight loss for the group was 5.6 kgms. The group mean specific gravity (determined by water densitometry) increased from 1.062 to 1.069 suggesting the loss of a tissue denser than adipose tissue. The leaner subjects appeared to lose leaner tissue. Net nitrogen loss also conformed to this pattern. The significance of NAAP and SCN space determinations was equivocal. Basal O_2 consumption, creatinine excretion and skinfolds decreased as anticipated. Control determinations of specific gravity were in agreement to within .001 and week-by-week changes indicated that this is a sensitive method. It is concluded that under the conditions of this experiment the tissue lost may be influenced more by initial body composition than by the diet.(Supported by grants from the Nutr. Found. and the Natl. Inst. of Arth. and Met. Dis. P.H.S.)

212. PLANT METABOLISM

1 *Biochemistry*

BACTERIAL PHOTOSYNTHESIS AND THE ASSIMILATION OF ACETATE VIA CITRAMALIC AND CITRIC ACID. D. I. Arnon, M. Losada*, A. V. Trebst* and S. Ogata*. Univ. of Calif., Berkeley, Calif.

The assimilation of labelled acetate by the photosynthetic bacterium Chromatium supports the theory that the light-dependent events in bacterial photosynthesis consist of ATP formation (Arnon, D.I., Nature 184, 10, 1959). Whole Chromatium cells assimilated acetate as the sole source of carbon only in the light. Cell-free preparations assimilated acetate either in the light, concurrently with photosynthetic phosphorylation, or in the dark when supplied with exogenous ATP; in both cases the assimilation was greatly increased by adding either pyruvate or oxalacetate. By trapping ATP with an added hexokinase system, either in light or in dark, acetate assimilation was abolished. Acetate assimilation depends on ATP and CoA for the formation of acetyl-CoA. In light or in dark, the dominant product in the oxalacetate series was citrate; in the pyruvate series, citramalate, formed by a condensation of acetyl-CoA with pyruvate. The citramalate and citrate condensations resulted in the formation of glutamate which was the dominant soluble product in short-term photoassimilation of acetate by whole cells. Associated with citramalate was a compound with an Rf corresponding to that of mesaconate. Chromatium extracts were also found to catalyze a rapid exchange of the carboxyl carbon of pyruvate with $C^{14}O_2$.

2 *Biochemistry*

EFFECT OF 2-CHLOROETHYLTRIMETHYLAMMONIUM CHLORIDE AND RELATED COMPOUNDS UPON PLANT GROWTH. N. E. Tolbert, Dept. Agr. Chem., Mich. State Univ., East Lansing.

Compounds of the structure $CH_2X\text{-}CH_2\text{-}N(CH_3)_3\cdot Cl$ are active as plant growth substances. Treated plants grow with shorter and thicker stems and darker green leaves than controls, but there is no decrease in weight. In assays with wheat and squash the effects are the opposite from those obtained with gibberellin and the action of these two growth substances are mutually antagonistic. In the most active derivatives the $CH_2X\text{-}CH_2$- group is $CH_2Cl\text{-}CH_2$-, $CH_2Br\text{-}CH_2$- or $CH_2{=}CH\text{-}CH_2$-. Active compounds are also formed when $CH_2X\text{-}CH_2$- is $CH_2I\text{-}CH_2$-, $CN\text{-}CH_2$-, $CH_3\text{-}O\text{-}CH_2\text{-}CH_2$-, $CH_2SH\text{-}CH_2$-, or $CH_2{=}CCl\text{-}CH_2$-. The trimethylammonium cation is necessary for activity and the ethyl carbon chain with the substituent X is optimal in length for maximum activity. The structure of these compounds suggest that they attach to an active site at two points--one for the trimethylammonium cation and one for the excess electrons at position X on the ethyl side chain. A $C^{14}H_2Br\text{-}C^{14}H_2$- derivative was not metabolized by either plants or algae. The compounds do not change the rate of $C^{14}O_2$ fixation in short-time photosynthesis experiments. Alterations among the products of $C^{14}O_2$ fixation by Chlorella appears to be caused in part by complex formation with one or more of these products, particularly glycolic acid.

3 *Biochemistry*

EFFECT OF CMU ON SPINACH CHLOROPLAST PHOTOPHOSPHORYLATION. André T. Jagendorf and Maurice Margulies.* McCollum-Pratt Inst., Johns Hopkins University, Baltimore, Maryland.

p-Chlorophenyl (1,1-dimethylurea) (CMU) inhibits O_2 evolution. Phosphorylation is inhibited, except that with pyocyanine or phenazine methosulfate (PMS) the inhibition is relieved by complete removal of O_2. Under micro-aerobic conditions CMU inhibition is also relieved by reducing PMS non-enzymatically, by white light or by ascorbate. CMU makes chloroplasts analogous to Chromatium chromatophores, where addition of a substrate relieves inhibition by O_2 (Newton, J.W. and Kamen, M.D., Biochim. Biophys. Acta 25, 462 (1957)). Both situations can be interpreted by considering that a) oxidizing and reducing equivalents are produced stoichiometrically by light, b) these equivalents are carried by molecules bound to the particulate structure and present in catalytic amounts only, c) for repeated photochemical acts to occur both the oxidizing and reducing equivalents must be removed continuously and stoichiometrically. Atmospheric oxygen can combine with reducing equivalents but will leave oxidizing equivalents bound to the initial carrier which therefore cannot function in a second light reaction. Further support is found in reversal of CMU inhibition of TPN reduction by adding ascorbate plus PMS.

4 *Biochemistry*

PHOTOPHOSPHORYLATION IN TRIS-TREATED SPINACH CHLOROPLASTS. Babette Stern (intr. by M. E. Hanke.) University of Chicago, Chicago, Ill.

It has been reported [J.B.C., 234, 2783 (1959)] that chloroplasts which have been treated with Tris buffer cannot reduce indophenol or ferricyanide, but have retained some of their capacity for causing anaerobic phosphorylation with cyclic cofactors. Further investigation of the effects of Tris treatment show that anaerobic photophosphorylation with phenazine methosulfate (PMS) is retained to a far greater extent than anaerobic phosphorylation with FMN or vitamin K_3. No precautions were taken to prevent the conversion of PMS to pyocyanine and the insensitivity of the system to inhibition by catalase and ethanol suggests that pyocyanine has been formed [R. Hill and D.A. Walker, Plant Physiol., 34, 240 (1959)]. The data suggest that pyocyanine may perhaps be the only one of the cofactors examined which elicits a truly anaerobic phosphorylation at rates equivalent to those obtained in the presence of O_2. With Tris-treated chloroplasts, O_2 is highly inhibitory to photophosphorylation mediated by PMS (pyocyanine), presumably because the reduced cofactor is oxidized by O_2 as well as by the internal photooxidant. The effects of 10^{-5}M CMU on photophosphorylation mediated by PMS and FMN in fresh chloroplasts are similar to the effects of Tris-treatment.

5 *Biochemistry*

PHOTOSYNTHETIC AND RESPIRATORY ENZYMES IN LEAVES. R. Smillie* and R. C. Fuller. Brookhaven National Laboratory, Upton, N.Y.

Enzymes were assayed in extracts of pea leaves at different stages of growth. As a function of leaf development, three distinct groups of enzymatic patterns were discernible. Group 1 included isocitric, malic, glucose-6-p, 6-phosphogluconic and lactic dehydrogenases, glutathione and glyoxalic reductases, PEP carboxylase, aconitase, enolase, phosphofructokinase, and malic enzyme. These enzymes did not appear to function in photosynthesis. Their peaks of activity were reached earlier than a number of photosynthetic activities and group 2 and 3 enzymes. Further, their activities (malic dehydrogenase excepting) at the developmental stage when maximum photosynthesis occurred, were less than that required by an enzyme actively participating in photosynthesis. Enzymes of group 2 appeared to be purely photosynthetic enzymes and as such were probably localized in the chloroplasts. They included RuDP carboxylase, TPN-linked glyceralderhyde-3-p dehydrogenase, and photosynthetic TPN reductase. Group 3 enzymes participated in both photosynthesis and respiration. Their total activity probably represented the sum of their activities in different parts of the cell. PGA kinase, DPN-linked glyceraldehyde-3-p dehydrogenase, triose-p isomerase, aldolase, and phosphoglucoisomerase were included in group 3. (Research carried out under the auspices of the U. S. Atomic Energy Commission.)

6 *Biochemistry*

SITE OF INHIBITION OF IODOACETAMIDE IN PHOTOSYNTHESIS AND RESPIRATION. Martin Gibbs and Nona Calo*. Dept. Biochem., Cornell Univ., Ithaca, N. Y.

It is generally assumed that glyceraldehyde-3-P dehydrogenase is the site of IAA inhibition in photosynthesis and respiration even though some concentrations of IAA strongly block O_2 evolution while permitting O_2 consumption to occur unimpaired. 5×10^{-5} M IAA inhibits (50%) CO_2 fixation by spinach chloroplasts. Since ATP, TPNH and O_2 synthesis are unaffected by 5×10^{-3} M IAA, the sole site of IAA inhibition in photosynthesis is apparently the carbon cycle. 5×10^{-5} M IAA does not affect the TPN-linked arsenate dependent and independent G-3-P dehydrogenases, transketolase, transaldolase, ribose-5-P isomerase, aldolase, neutral and alkaline fructose 1,6-diP phosphatase and ribulose diP carboxylase of the spinach chloroplast. Phosphoribulokinase, an enzyme concerned only with photosynthesis, is inhibited in this range of IAA. It is concluded that low concentrations of IAA block this phosphorylation thus halting photosynthesis but have no effect on plant respiratory enzymes. IAA poisoned plants, however, do not accumulate ribulose-5-P. (Supported by grants from the National Science Foundation and Atomic Energy Comm.)

7 *Biochemistry*

LIGHT-INDUCED ATP-HYDROLYSIS IN ANABAENA VARIABILIS AND SPINACH PREPARATIONS. Barbara Petrack (intr. by Moses Kunitz). The Rockefeller Institute, New York, N. Y.

A photophosphorylating cell-free preparation from blue-green algae has been described (Federation Proc., 18, 302, 1959). Although with this preparation 3.3×10^{-4} M stimulates, 1.3×10^{-2} M of glutathione (GSH) blocks photophosphorylation and will cause a light-dependent release of Pi from ATP in the presence of phenazine methosulfate (PMS) and Mg^{++}. The maximum rate of hydrolysis obtained with PEP-pyruvic kinase as a feeder is approximately 100 μM/hour/mg. chlorophyll. Di-iodohydroxy benzoyl butyrate which, at 5×10^{-5} M completely uncouples photophosphorylation, also inhibits ATP-hydrolysis. Spinach chloroplasts behave like algae preparations, but it is necessary to increase the concentration of GSH to 6.67×10^{-2} M; cysteine is usually more effective. The PMS may be replaced by FMN. In contrast to the algae system, both the photophosphorylation and ATPase of spinach chloroplasts are inhibited by NH_4^+, indicating an interrelation between these two activities. It is tempting to assume that the ATP-hydrolysis may reflect a partial reversal of the pathway of photophosphorylation. (Supported by a fellowship from the Damon Runyon Memorial Fund for Cancer Research.)

8 *Biochemistry*

COUPLED PHOTOREACTIONS OF CHLOROPLASTS. Leo P. Vernon and Waldo S. Zaugg.* Brigham Young University, Provo, Utah.

Freshly prepared chloroplasts from Phytolacca americana leaves have the ability to photoreduce the dye indigo carmine (IC) under anaerobic conditions. Addition of ascorbate, alone or with 2,6-dichlorophenolindophenol (DPIP) inhibits the reaction. Chloroplasts lose this activity upon aging. Addition of ascorbate and DPIP to aged chloroplasts restores the activity. The loss of the ability to directly photoreduce IC and the emergence of the requirement for ascorbate-DPIP parallels the loss of oxygen evolution capacity. With fresh chloroplasts, the presence of .1 M. NH_4 ion or 10^{-5} M. DCMU inhibits IC photoreduction, which is restored by addition of ascorbate and DPIP. Qualitatively similar results are obtained with DPN and TPN (with photosynthetic pyridine nucleotide reductase). It is concluded that with aged chloroplasts, which do not evolve oxygen, the photoreduction of IC requires ascorbate to remove the oxidizing equivalents produced in the chloroplast by light. With fresh chloroplasts this would not be necessary. The results obtained with the herbicide DCMU are consistent with the view that this compound inhibits the oxygen evolution system, since its presence with fresh chloroplasts makes them behave as the aged chloroplasts do.

9 *Biochemistry*

PARTICIPATION OF O_2 AND PEROXIDE IN PHOTOPHOSPHORYLATION ELICITED BY FMN AND VITAMIN K_3. T. Nakamoto and B. Vennesland. University of Chicago, Chicago, Illinois.

When FMN and K_3 are added to fresh spinach chloroplasts in sufficient amount to give maximum rates of photophosphorylation under anaerobic conditions (H_2), added catalase with ethanol inhibits the reaction. Among the possible interpretations, the following two alternatives are favored: either (1) catalase-ethanol can "trap" an oxygen precursor, or (2) the systems are not "truly anaerobic" even though O_2 is absent from the gas phase, but involve cyclic O_2 production and reduction to H_2O_2 with utilization of H_2O_2 as an oxidant to complete the cycle. Results of O^{18} studies show that the O_2-dependent photophosphorylation elicited by low concentrations of K_3 or FMN is associated with a rapid O exchange at a rate commensurate with the rate of photophosphorylation. Addition of catalase with ethanol to these systems gives no inhibition of photophosphorylation but results in net O_2 uptake at a rate which suggests that 1/3 to 1/2 of the O_2 uptake involves reduction of O_2 to H_2O_2. When O_2 tension is rate-limiting for photophosphorylation, added peroxidase stimulates strongly with both FMN and K_3, but added catalase stimulates only with FMN. Another difference between the two cofactors is the higher sensitivity of the FMN-linked reaction to hydroxylamine.

10 *Biochemistry*

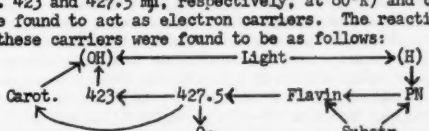
CAROTENOIDS OF EUGLENA GRACILIS (Z STRAIN) N. I. Krinsky and T. H. Goldsmith*, Biological Laboratories, Harvard University, Cambridge, Mass.

The nature of the stigma (eye-spot) of motile forms of various algae has never been adequately described. As its color indicates the possibility of carotenoids, we have investigated the nature of the carotenoid pigments of a number of strains of the green alga, Euglena gracilis. The Z strain has proved most interesting in this respect. Cells from a 96-hour culture were harvested, dried with anhydrous sodium sulfate and extracted with acetone and alcohol. After saponification, the pigments were transfered to petroleum ether and chromatographed on alumina. The following pigments were identified by a combination of chromatographic, spectral and chemical behavior. β-carotene, γ-carotene, echinenone, cryptoxanthin, OH-echinenone, antheraxanthin and a tri- or tetra-OH-α-carotene epoxide. An artifact, with slightly more polar properties, was formed from antheraxanthin during the extraction. Antheraxanthin, plus its artifact, account for over 80% of the total carotenoid pigment in this strain. As with other strains of Euglena gracilis, neither astaxanthin nor astacene could be detected. These findings will be discussed in relation to the stigma pigment(s).

11 *Biochemistry*

PATHS OF ELECTRON TRANSFER IN PHOTOSYNTHETIC BACTERIA. Mitsuo Nishimura (intr. by M. R. Ehrenstein), Johnson Foundation, University of Pennsylvania, Philadelphia, Pa.

The paths of electron transport in the photosynthetic and respiratory redox chains were studied in photosynthetic bacteria. In R. spheroides and R. rubrum two heme proteins (abs. max. 423 and 427.5 mμ, respectively, at 80°K) and carotenoids were found to act as electron carriers. The reaction sequences of these carriers were found to be as follows:



R. rubrum chromatophores showed absorption decreases at 387 and 604 mμ and increase at 434 mμ by illumination (cf. Smith & Baltscheffsky, J. Biol. Chem. 234: 1575 (1959). These locations of maxima did not change when the sample was cooled to 80°K. In Chromatium, a temperature-independent photochemical oxidation of a c-type cytochrome was observed (to 80°K) (Proc. Nat. Acad. Sci. in press). At room temperature, at least two groups of cytochromes (abs. max. 420 and 428 mμ, respectively, at 80°K) were found to react as electron carriers. The former group might contain more than one component (cf. Olson & Chance Arch. Biochem. Biophys. in press). This work was performed in collaboration with Dr. Britton Chance.

12 *Biochemistry*

FURTHER STUDIES ON OXIDATIVE PHOTOSYNTHETIC PHOSPHORYLATION. D. W. Krogmann (intr. by M.V. Buell). University of Chicago, Chicago, Ill.

The studies to be reported are concerned with the mechanism of photophosphorylation elicited by the addition of trichlorophenol indophenol to spinach chloroplasts. As previously reported, this dye system appears to be unique in that molecular oxygen is required for the occurrence of photophosphorylation, regardless of dye concentration. When the dye is acting catalytically and O_2 evolution is not inhibited, there is no appreciable net consumption or production of oxygen during the phosphorylation reaction. With the help of O^{18}, however, vigorous and nearly equal rates of consumption and evolution of O_2 by this phosphorylating reaction system have been observed. While this oxygen exchange reaction is dependent on light, the presence of trichlorophenol indophenol and undenatured chloroplasts, it is not obligately coupled to the synthesis of ATP. The rate of O_2 exchange is commensurate with the best rates of ATP synthesis achieved with this system. The relation of this O_2 exchange to the Mehler reaction will be discussed. Data will be presented which suggest that pyridine nucleotides may serve as a substitute for oxygen in this system.

213. PORPHYRINS

1 *Biochemistry*

MAGNESIUM PORPHYRINS IN CHLOROPHYLL BIOSYNTHESIS. S. Granick, Rockefeller Institute, New York.

Intermediates previously reported were protoporphyrin (PROTO), Mg protoporphyrin (Mg PROTO), and Mg vinyl pheoporphyrin-a_5 (MgVP) (Harvey Lectures 1948-49). Mg PROTO monomethyl ester now has been found in Chlorella # 60-A, and identified as being the monomethyl ester by a quantitative colorimetric method specific for methanol. A PROTO monoester is formed in a Euglena preparation fed δ-amino levulinic acid (δAL). When cut ends of shoots of etiolated barley are immersed in 0.01M δAL for a day, mainly MgVP is formed. If δAL and αα-dipyridyl are fed, the yield is double; about 40% of the δAL is converted to porphyrins, mainly MgVP with smaller amounts of PROTO, Mg PROTO and Mg PROTO monoester. These and previous results support the hypothesis that chlorophyll biosynthesis proceeds via PROTO → Mg PROTO → Mg PROTO monomethyl ester ⇢ MgVP ⇢ chlorophyll. (Supported in part by NIH RG-4922).

2 *Biochemistry*

DIURNAL CONCENTRATION OF SOYBEAN LEAF CHLOROPHYLLS. James L. Wickliff* and Sam Aronoff. Iowa State Univ., Ames, Iowa.

Controversy exists concerning the diurnal constancy of chlorophyll concentration in leaves. Using plants under greenhouse conditions, special care was given to methodology. Leaf punches of mature (non-expanding) trifoliate leaves of identical age were extracted by homogenization in ten Broeck tubes with 80% aqueous ethanol, the pigments in the centrifuged solution being quantitized spectrophotometrically. The method results in a standard deviation of 1.40%. Two-hr. sampling over a 24-hr. period (10 punchos/samples; samples in duplicate)were made for proximal, medial and distal parts of leaves. Combined data for the three leaf regions showed all values within 2.5% of the mean. Taken separately, the proximal region has a statistically significant max. at 0800, the distal at 1200, while the medial has a min. at about 1200 hrs. The summed values appear to have max. at 0800 and 2100 hrs., although these lie within the span of a 0.95 confidence coefficient. For any one region diurnal chlorophyll concentration does not exceed 5%. (Supported by a National Science Foundation grant).

3 *Biochemistry*

STUDIES ON THE METABOLISM OF BIS-(2-IODOETHYL-I^{131}) DEUTEROPORPHYRIN-H^3 (BID) IN TUMOR BEARING MICE. K. I. Altman and K. Salomon. Univ. of Rochester, School of Medicine and Dentistry, Rochester, N. Y.

Double labeling of this porphyrin was used in order to elucidate certain aspects of the metabolic degradation of this compound. The ratio of I^{131}/H^3 in the preparation injected was approximately 1.2. Approximately 30 μc. of tritium and approximately 1 μc. of I^{131} were injected intraperitoneally into each mouse. Porphyrins and bile pigments were extracted from livers, tumors, and several other organs, and their radioactivity and chemical concentration determined. The bile pigment fraction of the liver was found to have a ratio of I^{131}/H^3 which was significantly greater than 1.2. The porphyrin fraction of the tumor was found to have a ratio of I^{131}/H^3 of 1.2 (identical with the ratio of BID injected). It is concluded that BID contained in the tumor retained its original specific activity for several days after injection. With respect to the bile pigment fraction of the liver, it is postulated that in the process of the metabolic degradation to bile pigments an exchange of tritium for hydrogen has taken place.

(This paper is based on work performed under contract with the United States Atomic Energy Commission at the University of Rochester Atomic Energy Project, Rochester, New York).

4 *Biochemistry*

FORMATION AND CHARACTERIZATION OF SOME NEW TYPES OF METALLOPORPHYRIN. Samuel Schwartz, Basil L. Loudas*, and Howard L. Dinsmore*. Department of Medicine, University of Minnesota Hospitals, Minneapolis.

Bonding of a single atom of metal to only the central nitrogens of a porphyrin molecule is believed to characterize all metalloporphyrins regardless of the preparative method used. While this may be generally true of metalloporphyrins formed in acetic acid solution, additional metal bonding to the propionic acids has been found to occur under suitable conditions in ammoniacal and other solutions. Such metal salts have been formed with copper, iron, cobalt, zinc, and other metals. A third type of metalloporphyrin which appears to have more than one molecule of porphyrin per atom of copper has also been prepared. The 3 types of metalloporphyrin are most readily distinguished by infrared spectrometry or by quantitative metal analysis. Their visible absorption maxima differ only slightly. Considerable variation is found in the solubility and in various physiological and biochemical properties of these different complexes.

214. PROTEINS, POLYPEPTIDES

1 *Physiology*

THE HETEROGENEOUS NATURE OF HOG PEPSIN AND PEPSINOGEN. Jordan Tang and Kuen-I Tang (intr. by Stewart Wolf.) Okla.Med.Research Inst. and Dept. of Med. and Biochem., Univ. of Okla., Okla.City

When the three materials: hog gastric mucosa extract; crude pepsin (1:10,000); and 2 X crystallized pepsin were chromatographed with Amberlite IRC-50 (Richmond, et al., Biochim. Biophys. Acta, 29, 453, 1958), four pepsin peaks were eluted at pH 3.7, 3.8, 4.2 and 4.4. These accounted for 95.6% of the original proteolytic activity. The patterns were consistent in runs of all three materials. With a hemoglobin substrate the pH optimum for the enzyme in the first three eluted fractions was 1.5. The pH optimum for the fourth was below 1. No gastricsin was found in the preparations from the hog. Thus, the patterns contrast with those of human gastric juice where only a single pepsin peak appeared (pH optimum 1.8) and a single peak for gastricsin (gastric pH optimum 3.0 to 3.2). Two peaks of pepsinogen were detected when an alkaline extract of hog gastric mucosa after dialysis at pH 8, was subjected to electrophoresis on raw potato starch block in Na-acetate buffer pH 7.0 (u = 0.1). Constant potential of 4.55 volts/cm were applied for 17 hours at room temperature. The two pepsinogens were 4 and 8 cm from the origin toward anode. (Supported by Research Grants A-1107 and A-1315 from NIH, PHS).

2 *Biochemistry*

THE EFFECT OF POLYANIONS ON THE ACTIVITY OF TRYPSIN. S. E. Kornguth* and M. A. Stahmann. Dept. of Biochemistry, Univ. of Wisconsin, Madison, Wisconsin.

The effect of polyanions upon tryptic activity was studied as a function of type of anion, concentration, ionic strength, molecular weight and substrate. At low concentrations poly-α-L-glutamic acid caused 96% inhibition of tryptic hydrolysis of denatured proteins or benzoylarginineamide, but tryptic activity was restored when the concentration was increased. The restored activity was considerably higher than the activity in the absence of the polyanions in some cases. Inhibition decreased as the ionic strength increased. The inhibition was a function of the ratio of enzyme to the total number of anions rather than to moles of polymer. Data suggest that polymers in which the anions are separated by the distance between residues of an extended polypeptide (polyglutamic acid, polycysteic acid or heparin) block tryptic activity by complexing with basic groups on opposite sides of the active site of the enzyme. Poly-γ-D-glutamic acid, which lacks this common spacing of anionic groups, showed no inhibition. Native albumin or globulin, the hydrolysis of which was not inhibited, may displace the polyanion or restore the enzymatically active configuration allowing hydrolysis in the presence of the polyanion; whereas denatured proteins and benzoylarginineamide cannot do this.

3 *Biochemistry*

ISOLATION OF PURIFIED PROTEIN WITH ALLERGIC ENCEPHALITOGENIC ACTIVITY FROM BOVINE SPINAL CORD. Elizabeth Roboz-Einstein, D. Robertson* and J. DeCaprio*, Univ. of Calif. Medical Center, San Francisco, Calif., and Stanford University, California.

Previously it has been established that the agent which causes allergic encephalitis is protein in nature (Kies, Roboz, Alvord, Fed. Proc. 15:938, 1956). It has been shown that collagen which was isolated in pure form is active (Roboz, Henderson and Kies; Kies, Alvord and Roboz, J. Neurochem. 2:254, 261, 1958), also that a salt-soluble preparation which does not contain collagen possesses high activity (Roboz, Henderson; Kies, Alvord, Symposium on Allergic Encephalitis, p 281, 293, Thomas, Springfield, 1959). Separation of these salt-soluble proteins and isolation of the purified active component has been accomplished by the following procedure: Lyophilized bovine spinal cord was extracted at -70° with acetone and petroleum ether. After removal of the inactive proteins by water and sodium acetate extraction, the cord was treated with 5% KCl in 5 successive operations. The extract was purified with Dowex 50 resin and with ammonium sulfate. Fractionation was carried out by chromatography on DEAE cellulose and continuous electrophoresis. From the original extract containing about 9 proteins, we have obtained a protein which moves towards the negative electrode and is well separated from others. This protein was found homogeneous and highly active. (Supported by grants from USPH and National Multiple Sclerosis Society).

4 *Biochemistry*

SOME EFFECTS OF UREA ON MYROTHECIUM CELLULASE. P. K. Datta, K. R. Hanson and D. R. Whitaker (intr. by J. F. Morgan.) Div. of Applied Biology, National Research Council, Ottawa, Canada.

Two derivatives of cellulose, a carboxymethyl cellulose and a cellulose sulfate of comparable molecular weight, show a profound influence of the nature of the substrate on the enzymatic activity of Myrothecium cellulase in the presence of urea. At pH 5.0, concentrations of urea up to 8 M. stimulate activity towards the former and inhibit activity towards the latter. Both effects are reversible. Its pH dependence suggests that the stimulatory effect of urea is associated with cleavage of hydrogen bonds between enzyme and undissociated carboxyl groups of the substrate. Enzyme in 8 M. urea at pH 4.90, both in the presence and absence of these substrates, shows a shift of about one mμ towards shorter wavelengths. Certain other changes in the spectrum are time dependent and appear to be light-scattering effects resulting from changes in enzyme-substrate aggregates.

5 *Biochemistry*

THE MODE OF ACTION OF BACTERIAL ELASTASE. Ines Mandl and Betty B. Cohen*, Columbia University, College of Physicians and Surgeons, New York, N.Y.

Last fall we reported a new bacterial enzyme capable of solubilizing fibrous elastin (cf. Abstr. 136th ACS meeting 38C). The new enzyme resembles but is not identical with pancreatic elastase. It has a lower pH optimum of 7.4, is inhibited by Zn, Cd and Hg and is unaffected by common salts and serum elastase inhibitors. By adsorption-elution techniques and electrophoresis pigment and peptidase activities have been removed from the crude ammonium sulfate precipitates, but unspecific proteinase is still present. The elastolytic enzyme does not attack synthetic peptide substrates susceptible to pancreatic elastase, various proline containing peptides or specific collagenase substrates. Enzymatic breakdown products obtained when the bacterial elastase acts on ligamentum nuchae elastin were investigated. The N-and C-terminal amino acids of the resulting peptides were identified by dinitrophenylation and hydrazinolysis. Two-dimensional paper chromatography and elution of the DNP-amino acids gave about equal amounts of glycine, alanine, valine and glutamic acid, somewhat less leucine, traces phenylalanine and negligible amounts of proline N-terminal. At the C-terminal only proline, alanine and leucine were present in significant amounts. (Supported in part by NONR 266(49) and USPH D349.)

6 *Biochemistry*

ON THE SPECIFICITY OF THE REACTION OF IODOACETATE WITH A HISTIDINE RESIDUE IN RIBONUCLEASE. George R. Stark*, William H. Stein, and Stanford Moore. Rockefeller Institute, New York.

Iodoacetate reacts with ribonuclease at pH 5.5 in aqueous solution to yield an inactive derivative in which the imidazole ring of a single histidine residue has been carboxymethylated (Gundlach, Stein, and Moore, J. Biol. Chem., 234, 1754 (1959); cf. also Barnard and W. D. Stein, Biochem. J., 71, 14P (1959) for reaction with bromoacetate). Under similar conditions, performic acid oxidized-ribonuclease has been found to undergo no reaction with iodoacetate. If the disulfide bonds are split by reduction with sodium borohydride in water at 50° in the presence of Versene, and the mixture subsequently dialyzed rapidly at pH 5, there is no reaction with histidine when iodoacetate is added at pH 5.5; carboxymethylcysteine and the methionine sulfonium salt are formed. When ribonuclease is dissolved in 8 M urea or 4 M guanidinium chloride at pH 5.5, the enzyme is inactivated at about the same rate as it is in simple aqueous solution, but the inactivation is paralleled by alkylation of methionine, not histidine, residues. Thus, the reaction of iodoacetate with a histidine residue in ribonuclease at pH 5.5 requires the integrity of much of the three-dimensional structure of the active enzyme.

7 *Biochemistry*

CHROMATOGRAPHIC PATTERNS OF ENZYMES IN DIFFERENT RAT TISSUES. Blake W. Moore (intr. by Ethel Ronzoni.) Washington Univ. School of Medicine, St. Louis, Missouri.

Protein, nucleic acid, and 10 enzyme activities were determined in fractions of soluble rat tissue proteins from liver, kidney, brain, skeletal muscle, serum, and red cells, chromatographed on DEAE-cellulose. Some enzymes showed multiple peaks of activity, the relative proportions of which were characteristic of each tissue. For example, glucose-6-phosphate dehydrogenase gave 4 major peaks in liver; only peak 1 was present in muscle or serum; only peaks 2, 3, and 4 in red cells; only 1, 2, and 3 in kidney; and only 3 and 4 in brain. The activity of 6-phosphogluconic dehydrogenase showed one sharp peak in the same position in all tissues and served as a marker. The protein patterns were also characteristic of each tissue.

XUM

8 *Biochemistry*

PREPARATION AND PROPERTIES OF PURIFIED INTRINSIC FACTOR. Leon Ellenbogen (intr. by B. L. Hutchings). Biochemistry Research Dept., Biochemical Research Section, Lederle Labs., American Cyanamid Co., Pearl River, N. Y.

A purified intrinsic factor (IF) preparation was prepared from hog pyloric mucosa by extraction, pH adjustment, ammonium sulfate precipitation, fractionation on DEAE-cellulose and ultrafiltration. The purified IF was active in the fecal excretion test and in the modified Schilling test at a level of 0.33 mg. Ultracentrifugal analysis revealed that more than 85% of the purified IF sedimented as one peak with a sedimentation constant of 0.5 S and had a molecular weight of approximately 5,000. Immunochemical studies of the purified IF with the Ouchterlony double diffusion technique revealed 2 bands with rabbit antisera. The purified IF contained 10% nitrogen, 5% blood group A substance and less than 3% glucosamine. It was void of tryptic or peptic activity. The vitamin B_{12} binding as measured by dialysis binding was 3 μg per mg. of IF and was considerably higher than other fractions of lower potency obtained during the fractionation.

9 *Biochemistry*

ACTIVITY OF ACID PROSTATIC PHOSPHOMONOESTERASE ON PHOSPHORIC ESTERS WITHOUT FUNCTIONAL SUBSTITUENTS. GUENTER BARTSCH (*), S.J. THANNHAUSER AND GERHARD SCHMIDT. BOSTON DISPENSARY AND DEPARTMENT OF BIOCHEMISTRY, TUFTS UNIVERSITY SCHOOL OF MEDICINE, BOSTON.

Prostatic phosphomonoesterase is one of the few hydrolases whose lack of qualitative specificity allows studies on series of model substrates regarding the influence of structural features other than those involving functional groups, on enzyme action. So far, hydrolysis of phosphomonoesters of homologous alcohol from methanol to pentanol, further of octanoland hexadecanol was studied. Hydrolysis rates at the respective pH-optima (which were very similar for all substrates) incressed strikingly from methyl to octyl phosphate, but decreased on further chain-lengthening to hexadecanyl phosphate. (Increase of the hydrolysis rates of alkyl phosphates between methyl and butyl phosphates with dialyzed liver extracts was reported by Bamann et al., Biochem. Z. 327, 136 (1955)). kM-values decreased from methyl to octyl phosphates. Kinetics for the lowest members deviated from unimolecularity although split product inhibition was negligible. Propyl phosphate was hydrolyzed much faster than allyl phosphate.

10 *Biochemistry*

STUDIES ON THE SPECIFICITY OF THROMBIN. Y. Levin*, J.E. Folk, Jules A. Gladner, and K. Laki*. Natl. Insts. of Health, Bethesda, Md.

Several peptide derivatives have been prepared for study of the specificity of thrombin. The following were prepared in this laboratory: gly.arg.gly.OEt.; Hip.arg.gly.OEt.; Hip.arg.Am.; phe.arg.Am.; gly.phe.arg.Am.; phe.gly.arg.Am.; val.arg.Am., and phe.val.arg.Am. Elongation of the peptide chain on the amino side of the thrombin-susceptible bond apparently does not affect the rate of hydrolysis since gly.arg.gly.OEt. is split by thrombin at the same rate as the corresponding arginine amides. Conversely, changes in structure on the carboxyl-side of the thrombin-susceptible bonds does influence the velocity of the hydrolysis. Blocking of the amino group results in an increase in rate of hydrolysis, e.g., Hip.arg.Am., and Hip.arg.gly.OEt. are split much faster than gly.arg.gly.OEt., phe.arg.Am., and val.arg.Am. Elongation of the peptide chain at the N-terminal has the same effect in accelerating hydrolysis, e.g., phe.gly.arg.Am.; gly.phe.arg.Am., and phe.val.arg.Am., are hydrolyzed much more rapidly than the corresponding phe.arg.Am., and val.arg.Am. Thrombin has also been found to exhibit wide esterase activity. Bz.lys.OMe. is hydrolyzed at one-fifth the rate of Bz.arg.OEt. Thrombin appears to have a pronounced esterase activity toward the esters of several other basic amino acids.

11 *Biochemistry*

ON THE MECHANISM OF ACETYL TRANSFER. K. Bruce Jacobson (intr. by G. D. Novelli.) Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tenn.

An enzyme from pigeon liver catalyzes the transfer of the acetyl group from one aromatic amine to another (Bessman, S. P., and F. Lipmann, Arch. Biochem. Biophys. 46:252, 1953). This activity requires a sulfhydryl agent but is inhibited by free CoASH. This inhibition is reversed by cysteine. Another inhibitor of acetyl transfer is amethopterin (10^{-5} M), which competes with the acetyl donor but not with the acetyl acceptor. A study of the reaction between p-nitroacetanilide and aniline established the foregoing points. In contrast to the previous report by Bessman and Lipmann that the equilibrium constant for the transfer was about one, the products of the reaction, acetanilide and p-nitroaniline, do not react to a measurable extent. This apparent contradiction was resolved when the electronegativity of the para substituent of the aromatic amine was considered. If the substituent is strongly electronegative, the acetanilide derivative is a better donor than when the substituent is less electronegative; thus the effectiveness of the substituent to activate the acetyl group decreases according to the series; NO_2, Cl, H, CH_3. Therefore, the acetyl group quite likely has to have a significant carbonium character when it is to be transferred.

12 *Biochemistry*

INHIBITION OF HUMAN HEART GLUTAMATE-OXALACETATE TRANSAMINASE. F. W. Barnes, Jr., C. C. Burch*, and S. D. J. Yeh*. Johns Hopkins Sch. Med. and Hosp. Women of Maryland, Baltimore, Md.

Inhibition of glutamate-oxalacetate transaminase by isoniazid (isonicotinic acid hydrazide) has been suspected to occur in part through interaction with pyridoxal phosphate. Unexpectedly, at higher concentrations of the coenzyme the rate of transamination between aspartate and α-ketoglutarate was found to be accelerated as a function of coenzyme concentration, although at all lower concentrations of coenzyme no effect on reaction rate was demonstrable. Furthermore at the higher coenzyme concentrations inhibition by isoniazid was found. A plot of reciprocal rates against reciprocal coenzyme concentrations showed that the inhibition was competitive. It appears that at higher concentrations coenzyme acts as substrate, probably competitively. In these ranges of coenzyme concentration the enhanced reaction rate is inhibited in a competitive fashion by isoniazid. No evidence was found for interaction of isoniazid and coenzyme. (Supported by a grant from U.S.P.H., National Institutes of Health).

13 *Biochemistry*

COMPARISON OF CHYMOTRYPSINOGENS α AND B. Beatrice Kassell and M. Laskowski. Dept. of Biochemistry, Marquette Univ. School of Medicine, Milwaukee, Wis.

The preparation of a chymotrypsinogen B essentially free of neo-chymotrypsinogens (< 0.1 mole of C-terminal tyrosine and leucine) has been achieved. It has the following terminal amino acids:

H_2N-Cys----------Leu-Ala-Asp(NH_2)-COOH.

C-terminal leucine, previously reported (Fed. Proc. 18, 259 (1959), decreased as purer preparations were obtained. α-chymotrypsinogen has the same terminal groups (Bettelheim, J. Biol. Chem. 212, 235 (1955); Desnuelle and Rovery, in Neuberger, Symposium on Protein Structure, p. 155, 1957; Pechere et al., J. Biol. Chem. 233, 1364 (1958). Total amino acid composition of the two chymotrypsinogens differs. Chymotrypsinogen B contains 5 arginine, 12 proline and 4 methionine residues per 25,000 M.W. (paper chromatography confirmed by chemical methods). α-chymotrypsinogen contains 4, 9 and 2, respectively (Wilcox, et al., J. Biol. Chem. 228, 999 (1957). Activation of chymotrypsinogen B by trypsin involves hydrolysis of the arginyl-isoleucine bond, the same as activation of α-chymotrypsinogen (cf. Neurath and Dixon, Fed. Proc. 16, 791 (1957). (Supported by the U.S. Public Health Service and the National Science Foundation.)

Biochemistry

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FRACTIONATION BY DIALYSIS; IMPROVED SELECTIVITY. J. Goldstein*, and L. C. Craig. Rockefeller Institute, New York.

Earlier work (J. Am. Chem. Soc., 79 3729 (1957); Symposium on Protein Structure, A. Neuberger, Ed., John Wiley & Sons, Inc., New York 1958, p.104) has shown that differential rates of diffusion through membranes not only offers a useful way of separating mixtures but also offers a way of characterization with respect to size and/or shape. Selectivity has been shown to be a function of the solvent and also of the porosity of the membrane. It can be increased greatly by choice of a membrane which will barely pass the solute of interest. For the required adjustment of porosity, mechanical stretching, acetylation and zinc chloride treatment have proven effective. Selectivity has also been increased by an improved cell design including an arrangement for stirring. For example, a cell giving a 50% escape time of 3 hrs. with ribonuclease (M. W. 13,600) has given 9 hrs. with lysozyme (M. W. 14,800). A membrane adjusted to pass 50% of plasma albumin, a protein of M. W. 67,000, in 7.5 hrs. has passed active transfer RNA (50%) in 3.6 hrs. in salt solution.

Biochemistry

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REACTION OF 2-AMINO-1-NAPHTHOL WITH BOVINE SERUM ALBUMIN (BSA). Sidney Belman*, Walter Troll, and Norton Nelson. Inst. of Industrial Medicine, New York Univ., New York City.

2-amino-1-naphthol(ANA), a urinary metabolite (Troll, W., Royce, J., and Nelson, N.; Fed. Proc. 18, 340 (1959), of the bladder carcinogen 2-naphthylamine, was reacted for 16 hrs. with BSA in the presence of hydrosulfite, pH 7. The brown conjugate (ANA-BSA), after dialysis, was purified by precipitation with acetone and resolution in pH 7 buffer. Conjugates of 1,2-naphthoquinone-4-sulfonate (NQS) with BSA and ε-aminocaproic acid (ECA) were also prepared at pH 7. The absorption spectra of the three conjugates were similar at pH 7 and pH 13. The change in spectra at pH 13 is attributed to the ionization of a phenolic hydroxyl with a pK near 12. These results indicate that the same compound is formed from ANA and NQS. This would be expected if ANA is oxidized under these conditions to 1,2-naphthoquinone which reacts with lysine amino groups by 1,4 addition to give 4-(lysine-amino)-1,2-naphthoquinone. At pH 7 NQS-ECA has a λ max. at 460 mμ, ε_M=4,480. Using this ε_M, NQS-BSA had 14 groups substituted, and ANA-BSA had 1.7. (Aided by a grant from National Aniline Division, Allied Chemical Corporation and by a grant from the Public Health Service.)

Biochemistry

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COMPARATIVE STUDIES ON NATIVE AND REDUCED-REOXIDIZED RIBONUCLEASES. F. H. White, Jr. (intr. by C. B. Anfinsen.) Natl. Insts. of Health, Bethesda, Md.

Reduction of the four disulfide bonds of ribonuclease (RNase) with thioglycolate or mercaptoethanol results in complete loss of enzymatic activity. Air-oxidation of the reduced enzyme causes regeneration of most of the native specific activity (White, J. Biol. Chem., in press). Chromatography of the native and reoxidized enzymes on carboxymethyl cellulose gave nearly identical patterns. The specific activity of the main component of reoxidized RNase was 80 to 100% of that of native RNase. The main chromatographic component of each enzyme was digested proteolytically. The digests were separated two dimensionally on paper, by a combination of chromatography and electrophoresis, to produce patterns of cyanide-nitroprusside positive peptides which did not vary significantly, suggesting that the reduced enzyme had reoxidized to form the same disulfide bonds that exist in native RNase. There appeared to be no differences in secondary and tertiary structures, as indicated by comparative studies on immunological specificity (performed by Dr. R.K. Brown), viscosity, optical rotation, and ultraviolet spectrum. The present evidence suggests a specific refolding of reduced RNase on oxidation, to produce a three dimensional configuration closely resembling that of native RNase.

Biochemistry

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THE EFFECT OF ANIONS AND OF pH ON ATP-CREATINE TRANSPHOSPHORYLASE ACTIVITY. Lafayette Noda and Taiichi Nihei*. Dept. of Biochemistry, Dartmouth Medical School, Hanover, N.H.

The observation that tripolyphosphate did not bind on the protein, ATP-Creatine Transphosphorylase (Creatine Kinase), has led us to re-examine some effects of the environment on the properties of the enzyme previously reported [J. Biol. Chem. 209, 191; 210, 65 (1954)]. The specific activity of the crystalline enzyme carefully prepared to avoid possible alcohol denaturation (slowly reversible to some extent) has been found to be about 70 units per mg under the standard conditions previously used. However, sulfate is an inhibitor. The peculiar maximum activity at ATP/$MgSO_4$ = 1 previously observed with increasing $MgSO_4$ is not observed when $MgAc_2$ is used (activity reaches maximum at ATP/$MgAc_2$ = 1 and remains constant with increasing Mg^{++}). Common inorganic ions all inhibit to varying degrees and the doubly or triply charged anions are apparently better inhibitors. Acetate at 0.05 M and glycine, glycylglycine or histidine at 0.2 M do not inhibit the reaction catalyzed in the direction of formation of creatine phosphate. The pH activity curve appears to be like a titration curve with pK about 6.5 - 7, depending on conditions, and does not show the peculiar buffer effects when acetate is the anion. Mg-tripolyphosphate, free ATP and adenosine are competetive inhibitors of Mg-ATP as substrate.

Biochemistry

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ION EXCHANGE CHROMATOGRAPHY OF PROLACTIN. R. David Cole. Dept. of Biochemistry, Univ. of Calif., Berkeley, Calif.

Chromatography of non-basic proteins on the carboxylic ion exchange resin Amberlite IRC 50 has been attempted using eluting buffers containing urea. In this way successful chromatography was accomplished in the case of the small, non-basic protein insulin (Cole, Fed. Proc. 18, 810, 1959). This work has now been extended to the pituitary lactogenic hormone, prolactin, which is more representative of proteins in general since it has a molecular weight of 25000.

Experiments were carried out using 0.13M phosphate buffers which were in the range of pH 5.8 to pH 6.2 and which contained urea from 6M to 8M. The chromatography of prolactin is much more sensitive to pH and urea concentration than the chromatography of insulin. Using 0.13M phosphate-8M urea at pH 6.0, the protein recovery was quantitative; and when partially purified hormone was studied, the resolution of active material was satisfactory. (Supported by grant A 2691 from the U.S. Public Health Service.)

Biochemistry

19

APPLICATIONS OF GEL FILTRATION IN STUDIES OF PROTEIN-METAL COMPLEXES. Philip E. Wilcox and Jozef Lisowski*. Department of Biochemistry, Univ. of Washington, Seattle, Wash.

Gel filtration through columns of cross-linked dextran (Sephadex G-25, Pharmacia) according to the work of Porath and Flodin offers a convenient and rapid method for investigating complex formation between proteins and small molecules or ions. Studies on the binding of heavy metals to serum albumin and to α-chymotrypsinogen and certain of its derivatives will be described. In contrast to earlier reports, Sephadex is not entirely inert toward proteins, especially basic proteins such as α-chymotrypsinogen. Substantial binding occurs between pH 3 and 6 at low ionic strengths. The use of dilute salt solutions for equilibrating the columns and as eluant permits the protein to pass through the columns unretarded. If the salt is ammonium formate, it may be removed from the protein fractions by lyophilization. Heavy metals are also bound to Sephadex under some conditions; ammonium formate buffers may be used to prevent such binding. The separation of the methylmercury complex of boving mercaptalbumin from excess methylmercury has been readily accomplished. Sephadex columns have also been used for studies of the reactions of heavy metals with the disulfide bonds of protein. (Supported by grants from the National Institutes of Health (H-3037) and the Rockefeller Foundation.)

XUM

20 *Biochemistry*

ELECTROPHORESIS OF SERUM ALBUMIN IN ACIDIC MEDIA. John R. Cann (intr. by Julia B. Mackenzie), University of Colorado Medical Center, Denver.

Bovine serum albumin undergoes two electrophoretically distinguishable types of interaction at acid pH's. The first type occurs only in media containing acetate buffer or other carboxylic acid buffers. The resulting electrophoretic patterns have been interpreted in terms of binding of undissociated buffer acid by the protein. According to this model the various peaks in the pattern should constitute a single, reaction boundary in which the protein gradient does not become zero between any two peaks. While this prediction has been realized under a variety of conditions, the gradient does become zero between the two rising peaks observed in certain media. We have now shown that this latter behavior is an artifact arising from mild convective disturbances in the Tiselius cell. The second type of interaction--the isomerization reaction of Aoki and Foster--also yields reaction boundaries as evidenced by the patterns obtained after prolonged electrophoresis with back-compensation and by the fact that only a single peak is obtained on electrophoresis at a sufficiently low field strength.

21 *Biochemistry*

THE FRACTIONATION OF INSULIN ON DIETHYLAMINOETHYLCELLULOSE. M. Volini and M. A. Mitz (intr. by W. F. White.) Research Division, Armour and Co., Chicago 9, Ill.

Heterogeneity in purified insulin preparations has been demonstrated by means of several criteria. The fractionation studies of Harfenist and Craig (J. Am. Chem. Soc. 74:3083, 1952), Fredericq (Ciba Found. Coll. on Endocrin. IX:89, 1956), and Timasheff, et al. (J. Am. Chem. Soc. 75:3121, 3124, 1953) have indicated that the component differences can be attributed to variation in a few chemical groups resulting in slight differences in net molecular charge. In the present work, purified insulin was separated into two biologically active components (Insulins I and II) apparently unlike those obtained previously. Fractionation was accomplished on the anion exchanger DEAE-cellulose, using stepwise elution with CO_2-H_2O solution and 0.1 M $NH_4H_2PO_4$. The two insulins were compared as to biological activity, solubility, crystallizability, qualitative amino-acid content, optical rotation, and electrophoretic mobility. They showed identical qualitative amino-acid content but displayed significant differences in physical configuration and in net charge. Their apparent interrelationship is that of native and partially denatured forms. It is postulated that they differ from the end products of other fractionation procedures in that they are composed of polymeric forms, each containing a single monomeric species.

22 *Biochemistry*

PEPTIDE-NUCLEOTIDE ASSOCIATIONS IN RAT LIVER. Clement . Weinstein*, S. Seifter and J.I. Berkman*. Long Island Jewish Hospital, New Hyde Park, N.Y., and Dept. of Biochemistry, Albert Einstein Coll. of Med., Yeshiva Univ., New York.

At a previous Federation meeting (1958) we reported that perchloric acid extracts of rat liver contained nucleotides associated with peptides and reducing substances. Similar fractions have been prepared by others from a number of microorganisms. In the present study extracts were made both with ethanol and perchloric acid, and fractions obtained after adsorption to and elution from Norit were studied for capacity to form hydroxamates and hydrazides. Eluates were found to yield positive hydroxamate reactions both at room and elevated temperatures. The hydroxamates were extracted into pyridine and their spectra studied. Hydrazides were also prepared using 1 M hydrazine at 50°, and were converted to dinitrophenylhydrazides by reaction with fluorodinitrobenzene. Individual DNP-hydrazides were separated by chromatography on paper and showed presence of amino acids after hydrolysis with 6 N HCl. The nature of compounds in the Norit eluate responsible for hydroxamate and hydrazide formation will be considered.

23 *Biochemistry*

SELECTIVE CHEMICAL CLEAVAGE OF PEPTIDE BONDS. Walter Schneider (intr. by Milton L. Scott.) Dept. of Biochemistry, Cornell Univ., Ithaca, N. Y.

We like to report the first use of anhydrous hydrofluoric acid in promoting acyl migration in N-acyl derivatives of α-amino, β-hydroxy compounds. This finding is of great significance since it has been previously reported (G.G. Katz et al. Arch. Biochem. Biophys. 63, 106 (1956)) that proteins can be dissolved in anhydrous hydrofluoric acid and recovered from this solvent without loss of biological properties or presumably irreversible changes in the structure of the molecule. Anhydrous hydrofluoric acid has therefore great advantages over concentrated sulfuric acid which, it has been reported, promotes acyl migration in proteins but also leads to irreversible changes in the molecule. We are now investigating the acyl migration of synthetic di- and tri-peptides of serine in anhydrous hydrofluoric acid. Paper electrophoresis experiments at pH 2.5 indicate that N ⟶ O acyl migration occurs in this solvent. Acylation under acidic conditions of the newly formed free amino group in the O-seryl peptides, as for instance formylation in formic acid acetic anhydride prevents the reverse O ⟶ N acyl migration. Thus the ester bond can be cleaved under mild conditions under which amide bonds are stable. (Supported by a grant to Dr. G. P. Hess by the Nat. Inst. of Health.)

24 *Pharmacology*

OCCURRENCE OF A COMPOUND IN BRAIN CONTAINING γ-AMINOBUTYRIC ACID AND HISTIDINE. J.J. Pisano*, J.D. Wilson*, L. Cohen*, and S. Udenfriend. Natl. Insts. of Health, Bethesda, Md.

Deproteinized brain extracts have been found to contain a substance which is adsorbed onto charcoal and which upon elution can be hydrolyzed by heating in strong acid or base to yield γ-aminobutyric acid (GABA). This combined form of GABA, (I), was not adsorbed by anionic resins but was retained by the cation exchanger, Dowex 50, H^+. When (I) was purified by chromatography and paper electrophoresis then hydrolyzed it was found to contain histidine in amounts equivalent to GABA. Comparison of (I) with synthetic γ-aminobutyryl-L-histidine (homocarnosine) revealed that; 1) both have identical mobilities on paper electrophoresis at a variety of pH values, 2) both are hydrolyzed by carnosinase at comparable rates, liberating equivalent amounts of GABA and histidine, 3) the time course of acid hydrolysis is the same for both, 4) fluorodinitrobenzene reacts with the amino group of GABA in both, 5) the elution time from 100 cm. Dowex 50 columns is the same for both. Although (I) has not yet been isolated in final purity these findings indicate that it is most likely homocarnosine. Enzymatic formation of the latter has been shown by Kalyankar and Meister (J.B.C. 234: 3210, 1959). Approximately 10 mg of (I) is present per kg of beef brain; hog and dog brain contain smaller amounts. (I) has not been found in other tissues.

25 *Immunology*

ON THE PHYLOGENETIC PATHWAY OF LENS PROTEINS. N. Manski, T.P. Auerbach* and S.P. Halbert. Dept. of Ophthalmology, Columbia Univ., New York.

A variety of vertebrate lens antisera were prepared in the rabbit by using a source of antigen, lenses from species representing different stages of evolution of the vertebrates. The reaction of these lens antisera with the soluble lens proteins from the different species was tested by immunoelectrophoresis. With anti-human or bovine lens sera all the mammalian lens proteins show the presence of 9-10 immunologically related components. The number of lens proteins in common in lenses of different species was found to parallel their evolutionary level. By absorption of anti-mammalian lens sera with lenses of other species, it can be demonstrated that antibodies, against lenses of species which are lower in the evolutionary scale are removed. These results indicate that during evolution some of the lens components which were developed at a lower stage were then transfered to higher species which retained these components throughout the evolution of the vertebrate. Appropriate absorbed anti-lens sera can be used for tracing the sequence of the appearance of each lens component in human and other lenses. Attempts to isolate these different components from the mammalian lens are under way.

215. PROTEINS, POLYPEPTIDES—Metabolism

1 *Biochemistry*

PLASMA IRON INCORPORATION INTO FERRITIN. A. Mazur, S. Green* and A. Carleton*. Cornell Univ. Med. College and New York Hospital, New York, N.Y.

Studies with rat liver slices, homogenates, extracts, and pure compounds have revealed the participation of ATP and ascorbic acid in the incorporation of plasma-bound iron into ferritin. Scorbutic guinea pigs show a marked decrease of ferritin iron in liver and spleen as well as lowered incorporation of administered plasma-bound Fe^{59} into these ferritins as compared with controls. Stimulation of anabolic reactions in liver and spleen of rats by injection of E. Coli or E. Coli endotoxin (lipopolysaccharide) leads to an increased incorporation of plasma Fe^{59} into ferritins of these tissues compared to that observed in controls. After endotoxin, liver and spleen show increased weight, RNA, DNA, protein, and QO_2; RNA:DNA ratios are increased. Increase in liver ferritin chemical iron after endotoxin administration is greater than increase in ferritin protein indicating that the observed stimulation of plasma iron incorporation into liver ferritin is not due to increased acceptor protein, but rather to an increase in utilization of energy sources for ATP synthesis and direct participation of the latter in the incorporation reaction. (Supported by a grant from N.I.H. [A-1655].)

2 *Biochemistry*

ACCELERATION OF OSTEOGENESIS BY CHONDROITIN SULFATE-TREATED DEMINERALIZED BONE. Martin Burger*, Burton S. Sherman* and Albert E. Sobel. Dept. of Biochemistry, Jewish Hospital of Brooklyn, N. Y.

EDTA-demineralized rat bones with and without 1% chondroitin sulfate (CS) treatment and other materials were implanted into standard defects drilled into the parietal bones of young albino rats. In the vast majority of cases, osteoblastic activity, with trabeculae of new bone extending from the margin of the host bone into the area of the wound, was present one week after operation. After one week, the process of osteogenesis was accelerated in those instances where CS-treated demineralized bone was implanted. Usually, only 6 weeks or less were required to effect almost complete repair using CS-treated demineralized bone, while untreated demineralized bone and fresh homogenous bone required several additional weeks to achieve the same result. It was also found that when CS-reprecipitated collagen was used as an implant, the wound was almost entirely healed with compact bone 8 weeks following operation, while NaCl-reprecipitated collagen produced only moderate repair in 8 weeks.

3 *Biochemistry*

SOURCES OF METHIONINE FOR LIVER PROTEIN SYNTHESIS. A. Wainer* and A. L. Koch. Univ. of Fla. School of Med., Gainesville, Fla.

Methionine for liver protein synthesis is derived from 3 major classes: 1) diet, 2) breakdown of rapidly turning over proteins, mainly liver and plasma proteins, 3) metabolically more stable proteins in peripheral tissues, mainly muscle protein. The 2nd and 3rd classes influence the incorporation of isotope into liver proteins in qualitatively different ways, referred to as "recycling" and "feed back," respectively. The quantitative contributions were determined by feeding completely synthetic diets containing an adequate concentration of S^{35} labeled methionine and no cystine to rats 2, 4, 8, and 16 days. R, the relative specific activity of methionine S^{35} in liver to that present in the diet was fitted to a 2 term exponential equation: $R = 0.733(1-e^{-0.153t}) + 0.267(1-e^{-0.0196t})$, where t is time in days. The rate const. for the 1st term (0.153/day) corresponds to an apparent half life of 4.5 days for liver proteins but includes the effects of recycling. Comparing this to Swick's value for the true half life (2.7 days) obtained from experiments in which no recycling component is present, it is calculated that the contribution of each source is as follows: 1) diet 32.1%, 2) recycling 41.2%, 3) feed back 26.7%. Data will be presented for cystine metabolism and for turnover of plasma proteins. (Supported by a grant from the National Science Foundation.)

4 *Biochemistry*

STUDIES ON THE BIOSYNTHESIS OF A PLASMA GLYCOPROTEIN. Jonas E. Richmond. Dept. of Biological Chemistry, Harvard Medical School, Boston, Mass.

Radiosulfate was found to be associated with the plasma glycoprotein fraction of rabbits within a minute after injection. This radioactivity was not lost on dialysis against H_2O for 96 hours. The radiosulfate that associated with the glycoprotein fraction in the first 1 to 20 minutes could be dissociated from the protein by dialysis against 0.15 M NaCl, Na_2SO_4 or other electrolytes for 48 to 72 hours; however, at times greater than 30 minutes a portion (less than 10%) of the radiosulfate associated with this protein was not lost by dialysis. This electrolyte non-dialyzable glycoprotein radiosulfate reached a maximum after 6 hours. Radiosulfate associated with the glycoprotein reversibly, in vitro. The dissociation of this radiosulfate was a function of the ionic strength and pH. Amino acids associated with this glycoprotein in vitro to varying extents. Following the injection of C^{14}-labeled amino acids, C^{14} rapidly appeared in this protein. This radioactivity was not all lost on dialysis against the unlabeled amino acid and electrolytes. Glucose associated with the glycoprotein in vitro in a form which was not readily dissociated by dialysis. The pattern of association of carbohydrates in vivo was somewhat similar to that observed with amino acids.

5 *Biochemistry*

EFFECT OF CORTICOIDS ON PROTEIN METABOLISM. Anthony A. Albanese, Edward J. Lorenze*, and Louise A. Orto*. Nutr. & Metab. Res. Div., Burke Foundation, White Plains, N. Y., and St. Luke's Conv. Hosp. Greenwich, Conn.

Metabolic studies were done on 11 males (21-76 yrs.) to quantitate and characterize the nitrogen catabolic effects of prednisone and prednisolone. The subjects were maintained on self-selected but stable dietaries which provided an average of 32 calories and 1.23 gm. of protein per kilo per day, and supported an average nitrogen balance of +3.7 gm. per day, during a control period of 2 weeks. In the succeeding 2 weeks when the subjects received 30 mg. per day of the test corticoids, analyses of the dietary showed an intake of 33 calories and 1.20 gm. of protein per kilo per day with an average nitrogen balance of -2.9 gm. per day. Thus, although the dietary was approximately isocaloric and isonitrogenous in the control and test periods, the administration of the corticoids was associated with a mean loss of 6.6 gm. of nitrogen per day. Chromatographic measurements of fasting blood amino acids showed a 25 per cent decrease in free plasma lysine (P greater than 0.01) during the corticoid test period. Observed changes in plasma methionine and threonine were not statistically significant.

6 *Biochemistry*

PROTEINASE OF HUMAN UTERUS. J. F. Woessner, Jr. and T. H. Brewer (intr. by G. T. Lewis.) Howard Hughes Medical Institute and University of Miami School of Medicine, Miami, Florida.

The ability of the involuting uterus to effect the breakdown of the collagen molecule (Fed. Proc. 18, 461, 1959) has prompted an investigation of the proteolytic enzymes of this organ. Crude extracts of normal and involuting human uterus produce optimum dissolution of uterine collagen at pH 3.4 and digest hemoglobin optimally at pH 3.0 - 3.6. Minimal activity is shown toward either substrate at pH values greater than 5.0. A proteinase responsible for the major portion of the hemoglobin digestion at pH 3.5 has been purified 30-fold by acid precipitation, negative gel adsorption and ammonium sulfate fractionation. This proteinase has no effect on benzoyl-L-argininamide, CBZ-L-glutamyl-L-tyrosine, or glycyl-L-phenylalanine amide. The ability to digest hemoglobin is unaffected by repeated dialysis, versene, cysteine, glutathione, ascorbic acid, p-chloromercuribenzoate, iodoacetate, Mg, Mn, Zn, Co, Ca, or ferrous ion. Cu, Pb, Hg, cyanide and diisopropylfluorophosphate are inhibitory. These characteristics appear to distinguish this proteinase from other mammalian cathepsins reported in the literature.

XUM

7 *Biochemistry*

AMINO ACIDS AND PROTEINS IN HYPERKERATINIZING RODENT SKIN. David L. Kirk and William G. Hoekstra (intr. by P. H. Phillips). Dept. of Biochemistry, Univ. of Wisconsin, Madison.

In an attempt to describe in biochemical terms the hyperkeratinization which follows topical application of certain aliphatic hydrocarbons, guinea pigs and hairless mice were treated with varying levels of n-hexadecane and their skins submitted to analysis at intervals thereafter. The progressive thickening of the epidermis was accompanied by an increase in protein per unit area--up to ten-fold that of control skin--and a decreased water content. The keratinous layer was observed to be more compact than that of control skin and had a higher affinity for acidic dyes. Chromatographic analysis of the free amino acids extracted from the epidermis revealed that, whereas the concentrations of most of the amino acids were relatively constant, arginine and its metabolites were present in far lower concentrations in the hyperkeratinized skin. This decrease seemed to be accompanied by a corresponding increase in the arginine content of the proteins of the epidermis. These observations indicate that the keratins formed in this condition have a higher arginine content than normal, which permits modified inter-peptide bonding and the formation of a more compact keratinous layer. Furthermore this increase is at the expense of the free amino acids of the skin, which results in decreased water-binding potential.

8 *Biochemistry*

ENZYMATIC HYDROLYSIS OF PROTEIN AMIDE GROUPS. M.J. Mycek* and H. Waelsch, Columbia Univ. and N. Y. State Psychiatric Inst.

The enzyme transglutaminase, has been shown to catalyze the exchange of the γ-amide groups of protein-bound glutamine with a variety of primary amines, as well as ammonia and the ε-amino group of protein-bound lysine. The reaction is accompanied by a liberation of ammonia which may exceed the amount of amine incorporated under some reaction conditions (M.J. Mycek, D.D. Clarke, A. Neidle, and H. Waelsch, Arch. Biochem. Biophys. 84:528, 1959). Also in the absence of added amine, considerable quantities of NH_3 are released which may be a consequence of the hydrolysis of the amide groups or cross-linking with free protein amino groups. When casein, β-lactoglobulin and insulin are acetylated or exhaustively deaminated, the enzymatic NH_3 release is comparable to that from the unmodified proteins. It, therefore, appears likely that the Ca^{++}-activated enzymatic reaction can effect the hydrolysis of protein-bound amide groups. That the same amide groups are involved in both the transfer and hydrolysis reactions is supported by the finding that enzymatically deamidated insulin shows a correspondingly smaller extent of enzymatically catalyzed amine incorporation. These findings suggest that the amide groups of protein-bound glutamine may not be metabolically inert, and raises the question as to the possibility of the enzymatic amidation of protein-bound glutamic acid.

216. PROTEINS, POLYPEPTIDES—Physicochemical Properties

1 *Biochemistry*

ARSENATE ACTIVATION OF PHOSPHOGLYCERIC ACID MUTASE. Jack C. Towne, (intr. by N.S. Radin), V.A. Research Hosp. and Dept. of Biochemistry, Northwestern Univ. Medical School, Chicago, Ill.

When the activity of crystalline yeast phosphoglyceric acid mutase (PGAM) is measured using the procedures of Towne, et al. (J.Biol.Chem.226,777(1957)) without added D-2,3-diphosphoglycerate (DPGA), an induction period of 25 minutes is observed before enzymic activity begins. This induction period is abolished by the addition of DPGA, and the rate of the reaction is proportional to DPGA concentration between 10^{-6} and 10^{-5} M. The induction period may be shortened by higher concentrations of PGAM. It was found that 0.00017 - 0.033 M arsenate reduces the induction period and enhances the reaction rate. The effect of arsenate is maximal at pH 6.5-7.0, which corresponds to the pH optimum for PGAM with this assay. Optimum activity occurs at 0.003-0.013 M arsenate. Arsenate does not enhance enolase activity; inhibition occurs at 0.033 M. The activating effect of arsenate can be demonstrated with PGAM isolated from rabbit skeletal muscle but not with PGAM isolated from wheat germ. The latter enzyme does not require DPGA for maximal activity. These observations indicate that muscle and yeast PGAM, despite different molecular properties, may have similar mechanisms of action; but wheat germ PGAM may act via a different mechanism of action.

2 *Biochemistry*

DIFFERENCES BETWEEN THE LACTIC DEHYDROGENASES ISOLATED FROM HUMAN HEART AND LIVER. J.S. Nisselbaum* and Oscar Bodansky. Division of Enzymology and Metabolism, Sloan-Kettering Institute for Cancer Research, New York, N.Y.

Lactic dehydrogenase (LDH) has been purified about 250-fold from human heart and from human liver. Enzyme from both organs has been obtained in crystalline form. Electrophoretic analysis indicates that the enzyme from heart migrates at a rate between albumin and α_1-globulins, and LDH from liver migrates at a rate between γ and β-globulins. Rabbit antiserum to human liver LDH completely inhibits the homologous enzyme, but does not inhibit the LDH from heart. Substrate-velocity relationships for pyruvate, DPNH, DPN and DPN analogs with LDH from both sources will be discussed.

3 *Biochemistry*

THE RESISTANCE OF CONALBUMIN AND ITS IRON COMPLEX TO PHYSICAL AND CHEMICAL TREATMENTS. P. R. Azari* and R. E. Feeney, Department of Biochemistry and Nutrition, Univ. of Nebraska, Lincoln.

Conalbumin, the iron-binding protein of egg white, forms a red-colored, strongly associated complex with 2 atoms of Fe^{+++}. This chromogenic capacity with iron is reported to be destroyed when the uncomplexed native protein undergoes even the mildest of physical or chemical treatments. Complex formation, however, has recently been reported to stabilize conalbumin to proteolysis and to thermal denaturation (Azari and Feeney, J. Biol. Chem., 232, 293 (1958)). These studies have now been extended to include other denaturing treatments (exposure to urea, high pressure, and organic solvents) and to chemical treatments (sulfite with $Cu(NH_3)_4^{++}$ and iodination). In all cases, the iron complex was more stable than the uncomplexed protein. It was possible, however, to modify the iron complex chemically without loss of chromogenic capacity as evidenced by changes in electrophoretic and solubility characteristics. This retention of the chromogenic capacity when the molecule is chemically modified is considered an example of stabilization of the molecule to denaturation as well as a possible protection of an active site to modification. (Supported by a grant from Natl. Insts. of Health.)

4 *Biochemistry*

THE EFFECT OF CERTAIN IONS ON BETA-GALACTOSIDASE ACTIVITY. F. J. Reithel and J. C. Kim*. Chemistry Dept., Univ. of Oregon, Eugene, and National Science Found., Wash., D. C.

This study was undertaken to determine what ions were required for maximum hydrolytic rate when various substrates were employed. The ions being considered were Mg^{++}, Na^+, K^+, and H^+. Hydrolysis data was obtained by colorimetry when ortho nitro phenyl galactoside was used, by enzyme spectrophotometric analyses when lactose was the substrate and by use of spectrophotometric polarimetry when beta methyl galactoside was investigated. It was found that homogeneous beta galactosidase from either ML 309 or K 12 strains of E. coli required Mg^{++} for maximum activity, that Na^+ was more effective than K^+ in the hydrolysis of ONPG, that K^+ was more effective than Na^+ in the hydrolysis of lactose and that the effectiveness of Na^+ or K^+ in the case of methyl galactoside depended on concentration. It is suggested that Na^+ & K^+ are bound to the enzyme in such a way as to affect the protein configuration at the active site. (Supported by a grant from the National Institutes of Health)

5 *Biochemistry*

PURIFICATION OF A PROTEOLYTIC ENZYME FROM A MARINE BACTERIAL SPECIES. J.M.Prescott and C.R.Willms(Introduced by H.O.Kunkel) Dept. of Biochem. & Nutr.,Texas Agr.Exp.Sta.,College Station.

One of the marine bacterial species isolated by Merkel and Traganza (Bact.Proc.,1958) has previously been shown to elaborate an active proteolytic system (Proc.Soc.Exp.Biol.Med., in press). A purified proteolytic enzyme was isolated from culture filtrates of this organism. Purification was accomplished by ammonium sulfate precipitation at 0.4 saturation, followed by adsorption on calcium phosphate gel and elution with phosphate buffer at pH 10.5. Active preparations thus obtained showed one peak in the ultracentrifuge and in the electrophoresis at two pH values. The enzyme was active against denatured hemoglobin and crystalline insulin, but did not hydrolyze benzoyl-L-argininamide or several other synthetic substrates tested; studies on substrate specificity are being continued. The enzyme was most active at pH values of 7.5 to 8.0, and exhibited the greatest stability in this range. Activity was stimulated by Co^{++} ions, but was inhibited by Hg^{++}, Fe^{+++}, Cu^{++} and Ni^{++} ions, and by cysteine, glutathione, and cyanide; p-chloromercuribenzoate was without effect. Sedimentation velocity measurements indicated a molecular weight of 55,000-60,000.

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6 *Biochemistry*

ACTIVE SITES PER MOLECULE OF ACETYLCHOLINESTERASE DETERMINED IN A HIGHLY PURIFIED PREPARATION. H. Claire Lawler (intr. by D. Nachmansohn) Depts. of Neurology and Biochemistry, College of Physicians and Surgeons, Columbia University, New York.

Purified acetylcholinesterase, prepared from electric eel tissue and consisting of a single homogeneous component on ultracentrifugal analysis, hydrolyses over 300 mmols acetylcholine per mg protein per hour. The molecular weight estimated by physical methods is of the order of 10 million. The number of substrate binding sites per molecule was determined with organophosphorous inhibitors such as diethoxy-phosphorylthiocholine and others. To eliminate errors due to non specific protein binding a strong competitive, reversible inhibitor, prostigmine, was used to protect the active centers, then the organophosphorous compound was added. After dialysis the active sites were titrated with the organophosphorous inhibitor. Their number per molecule was found to be very high.

7 *Biochemistry*

PURIFICATION AND CHARACTERIZATION OF PORCINE CARBOXYPEPTIDASE B. J. E. Folk, J. A. Gladner, W. R. Carroll* and K. A. Piez. National Institutes of Health, Bethesda, Md.

Carboxypeptidase B has been defined as a proteolytic enzyme capable of rapidly hydrolyzing peptide bonds to release certain carboxyl-terminal basic amino acids. The zymogen has been partially purified from powders of bovine pancreas gland (Folk and Gladner, J.Biol.Chem. 231:379, 1958). This report presents the method of purification of active carboxypeptidase B from powders of autolyzed swine pancreas glands and outlines some of the chemical and physical properties of the molecule. The enzyme behaves as a pure and homogeneous protein with respect to ion-exchange chromatography, sedimentation analysis and moving boundary electrophoresis. The molecular weight (sedimentation and diffusion) is 34,300. The enzyme contains 1 gm. atom of zinc per mole which is both a structural and functional component. The amino acid composition is somewhat similar to that of carboxypeptidase A, the main differences being in the lower content of serine and in the higher content of tryptophane, amide ammonia and sulfur-containing amino acids.

8 *Biochemistry*

CERTAIN PROPERTIES OF α_s-CASEIN. David F. Waugh, Martha Ludwig*, J. Morton Gillespie*, Jean Garnier*, Elizabeth S. Kleiner*, and Robert W. Noble, Jr.* Dept. of Biology, Massachusetts Institute of Technology, Cambridge.

The caseins in the micelles of milk are isolated by centrifugation and brought into solution at pH 7 by sequestering calcium with citrate. After removal of calcium citrate by dialysis, the α_s-κ-casein complex is split at 0-5°C by adding calcium chloride at pH 7 to give a 0.25 M concentration. Crude α_s-casein is purified by three cycles involving precipitation from 0.1% protein solution at 5-10°C with calcium ions. The resulting α_s-casein contains 0.5% sulfur but gives no test for disulfide groups, in contrast to κ-casein which contains cysteine disulfide. α_s-casein appears electrophoretically homogeneous over a wide pH range and has a mobility of 7.05 at pH 2.35 (descending). It contains 1.1% phosphorus, 15.61% nitrogen and the orcinol test for carbohydrate is negative. The optical density of a 1% solution using a path length of 1 cm and λ = 2800 A is 10.66 at pH 7. (Supported by Grants from The National Science Foundation and The National Institutes of Health).

9 *Biochemistry*

NUCLEAR MAGNETIC RESONANCE STUDIES OF PROTEINS. Arthur Kowalsky* and P. D. Boyer. Dept. Physiol. Chem., Univ. of Minn., Mpls., Minn.

A preliminary survey of the proton magnetic resonance spectra of various proteins at 56.4 Mc. in D_2O with (trimethylsilyl)carbinol as an internal reference has been made. The spectrum of native ribonuclease shows 6 peaks. In urea these become sharper and a new peak appears at the position of the methionine methyl group. Eleven narrow peaks are observed with oxidized ribonuclease; tyrosine, phenylalanine and histidine are clearly resolved. Small shifts are apparent in the position of various peaks under different conditions. The spectrum of reduced cytochrome c is similar to that of the oxidized form but the peaks are narrower and more resolved. The spectrum of pepsinogen shows a broadening when compared to that of pepsin. Aldolase shows one broad unresolved line which in urea is split into 4 prominent peaks. Native chymotrypsin in 0.1 M KCl shows 3 broad bands. In urea these are resolved, each showing several components. The spectrum of beta-lactoglobulin is also slightly more resolved in the presence of urea. The poorly resolved spectra may be explained by association of the protein molecules to large aggregates with consequent slowing of the tumbling rate or more likely by a decreased freedom in the internal motions of the components of the protein molecule.

10 *Biochemistry*

CONFIGURATIONAL CHANGES OF TRYPSINOGEN IN ALKALINE SOLUTION. L. B. Smillie and C. M. Kay (Intr. by Jules Tuba). Univ. of Alberta, Edmonton, Canada.

The ionization behavior of the tyrosine residues of proteins in alkaline solution can be followed by measurements of the molar extinction in the ultraviolet region of the spectrum. Titration curves have been constructed at 10°, 25° and 37°C. for trypsinogen in the pH range 8 to 13. These curves indicate that 4 of the 9 to 10 tyrosine residues are free to ionize with pK_a of 10.5 (at 25.0°C.). The remainder are free to ionize only upon disruption of the native structure of the molecule at high pH. Reversible changes in the sedimentation coefficient and weight intrinsic viscosity of trypsinogen at lower pH have been interpreted as indicating a reversible configurational change since there is no alteration in molecular weight as measured in the light scattering photometer. It is concluded that a reversible configurational change in the trypsinogen molecule in alkaline solutions is followed at higher pH by a molecular unfolding during which the masked tyrosines become available for ionization. (Supported by a grant from the National Research Council of Canada).

XUM

11 *Biochemistry*

THE DENATURATION OF THYROGLOBULIN. H. Edelhoch and H. Metzger*. Natl. Insts. of Health, Bethesda, Md.

The denaturation of thyroglobulin in neutral and alkaline regions has been studied by solubility criteria. In the neutral range a trivial pH dependance of the first order velocity constants was observed. At pH's 11-12, however, the rate varied with the 3.4 power of the hydrogen concentration. The heat of activation declined by about 1/2 in this pH range. It was 165 kcal. at pH 8.1 and 80 kcal. at pH 11.8. Extensive conversion of native 19S thyroglobulin into species with sedimentation rates of 12S and 15S without concomittant solubility changes was obtained on increasing the temperature at neutral pH's. In the temperature range of denaturation (above 53°) no new molecular species were formed. At pH 12 the principal component was a molecule with an 8S sedimentation constant. Viscosity and optical rotatory measurements on aqueous solutions containing thyroglobulin denatured in both pH zones showed only small changes. However, major changes in viscosity and optical rotation were obtained in either 6M guanidine or 9M urea. In many ways the data resembles that obtained by Eisenberg and Schwert (1951) for chymotrypsinogen. The high heats (and entropies) of activation do not seem to be associated with any gross unfolding of the polypeptide chain and therefore other possible mechanisms of denaturation must be considered.

12 *Biochemistry*

PHYSICOCHEMICAL PROPERTIES OF ALPHA CRYSTALLIN FROM BOVINE LENS. Salil K. Niyogi* and Virgil L. Koenig. Northwestern University Medical School, Chicago Ill.

Alpha crystallin (AC), isolated from bovine lens and purified by repeated isoelectric precipitation, showed some interesting properties. AC gave one component in the ultracentrifuge when dissolved in 0.1M, 0.2M, and 0.3M NaCl (pH 5.8), 1.0 M NaAcetate (pH 7.4), 1.0 M NaAcetate (to pH 8.6 with 0.1 M NaOH), and 0.033 to 0.200 M Na_2HPO_4 (pH 9.1). Two components were present when AC was dissolved in solutions of NaCl-HCl, pH 2.0, $\Gamma/2$ 0.05 and 0.10. One component only was present in electrophoretic analyses in veronal buffer, pH 8.6, $\Gamma/2$ 0.1 and NaCl-HCl buffer, pH 2.0, $\Gamma/2$ 0.1. AC, showing two components in the ultracentrifuge when dissolved in NaCl-HCl, pH 2.0, $\Gamma/2$ 0.05, gave only one component when pH was adjusted to 4.0, 7.7, and 9.2. When pH was readjusted to 2.0, two components again appeared, indicating an apparent reversible dissociation. S^0_{20} of AC at pH 9.1 is 18.82, while at pH 2.0, values are 8.28 and 1.45 for the two components. Light-scattering measurements indicate variation of molecular of AC with ionic strength at pH 9.1. The increase in molecular weight at higher ionic strengths indicates apparent aggregation.

13 *Biochemistry*

PURIFICATION AND PARTIAL CHARACTERIZATION OF A VIRAL HEMAGGLUTINATION INHIBITOR FROM HUMAN ERYTHROCYTES. Ralph H. Kathan*, Richard J. Winzler, and Clarence A. Johnson*, Dept. of Biol. Chem., Univ. of Ill., Coll. of Med., Chicago, Ill.

A potent glycoprotein inhibitor, believed to be identical with the viral receptor site, has been isolated from type O Rh positive human red cells. The cells are hemolysed, the ghosts washed as free of hemoglobin as possible, the pH of the stromal residue adjusted to 8 and extracted with hot phenolic solution. After cooling, the aqueous layer is decanted, dialysed, and finally lyophilized. The slightly pink powder so obtained is extracted with a chloroform-methanol-water mixture. The upper aqueous layer, following dialysis and lyophilization, yields a white preparation possessing high inhibitory titer against all available strains of influenza virus. Physicochemical data, including that from ultracentrifuge and electrophoretic studies, suggest that the material is reasonably homogeneous. Upon complete hydrolysis the following amino acids were identified chromatographically: aspartic acid, glutamic acid, lysine, glycine, serine, alanine, proline, valine, and phenylalanine. Less rigorous hydrolyses indicated the presence of a hexose, two hexosamines, fucose, and sialic acid. Inhibitory activity was destroyed by trypsin, periodate, influenza virus, and the receptor-destroying enzyme of V. cholerae. (Supported by Grant #2520 from the USPHS.)

14 *Biochemistry*

EFFECTS OF ETHYLENEDIAMINETETRAACETIC ACID (EDTA) AND Ca^{++} ON THE CYSTEINE ACTIVATABLE PROTEINASE OF CL. HISTOLYTICUM. James D. Ogle and Paul L. McHenry (intr. by Dana I. Crandall.) Dept. of Biological Chemistry, Univ. of Cincinnati College of Medicine, Cincinnati, Ohio.

The hydrolysis of L-arginine methyl ester (AME) by the cysteine activatable proteinase (CAP) of Cl. histolyticum was inhibited by 10^{-4} M EDTA. This inhibition was pH dependent, 100 per cent at 8.5, 39 per cent at 7.6 and 29 per cent at 6. Inhibition was not reversed by increase in substrate concentration. If CAP was incubated with EDTA for 5 min. prior to addition of the substrate, inhibition was reversed only by Ca and to a lesser extent by Mn or Mg ions. Likewise when CAP was dialyzed against EDTA, only Ca, Mn, or Mg ions were effective in reversing inhibition. The metal could not be removed from the enzyme by low pH dialysis. CAP was rapidly and irreversibly denatured by incubation (>5 min.) with EDTA at 37°. This denaturation was not prevented by addition of substrate, Ba^{++} or Li^{+} during treatment with EDTA. When CAP was treated with p-chloromercuribenzoate to bind sulfhydryl groups and then dialyzed, only Ca, Mn or Mg ions reactivated the enzyme after removal of the mercuribenzoate group. The effects of other inhibitors, activators and metal ions will be discussed.

15 *Biochemistry*

STUDIES ON PHYSICAL CHEMICAL PROPERTIES OF SPERM WHALE MET-MYOGLOBIN. Frank R. N. Gurd and Esther Breslow*. Cornell University Medical College, New York 21, N. Y.

The titration behavior, Cu(II)-binding properties, and ability of metmyoglobin (Metmb) to catalyze the hydrolysis of p-nitrophenyl acetate (NPA) were studied at 25° in 0.16 M KCl. The phosphate-crystallized protein (kindly supplied by J. C. Kendrew) was deionized by exhaustive dialyses against KCl and glass distilled water. At pH 4.5 Metmb underwent a partially reversible denaturation accompanied by proton uptake and by a profound change in heme spectrum (c.f. hemoglobin). A similar but non-identical reversible change in spectrum can be evoked at higher pH values in the presence of 0.001 M $CuCl_2$. In Cu-binding studies Metmb exhibited a sharp uptake of 6 Cu ions per mole which was essentially complete at an unbound Cu concentration of 0.001 M ; at higher Cu concentrations a very gradual increase in bound Cu was observed. A marked decrease in solubility was found to accompany the binding of the fourth mole of Cu whereas the spectral change appeared to coincide with the binding of the sixth Cu ion. Changes in pH accompanying the binding suggest that the Cu-binding sites normally are fully protonated at pH 7. Addition of Cu to Metmb at pH 7 reduced the catalytic activity towards NPA, as did adjustment with HCl to pH 6.1. (Supported by grants from the National Institutes of Health, H-2739 and HF-8049.)

16 *Biochemistry*

IMMUNOCHEMICAL STUDIES ON RHODOSPIRILLUM HEME PROTEIN. Joseph A. Orlando* and Martin D. Kamen. Grad. Dept. of Biochemistry, Brandeis University, Waltham, Mass.

The unique properties of Rhodospirillum heme protein (RHP), a variant of cytochrome c, could possibly result from modifications produced during the purification procedure. Specific rabbit antiserum for purified RHP was prepared and characterized by double diffusion in agar and precipitin analysis. With the homologous antiserum it was possible to distinguish partially and completely denatured forms of RHP from the purified protein. Immunochemical analysis of crystalline RHP, and partially purified extracts of R. rubrum prepared by such mild procedures as lysozyme lysis, sonication, and freezing and thawing gave serological responses very similar to those of the purified RHP. Gel diffusion experiments indicated a small, but quantitatively insignificant reaction between crystalline R. rubrum cytochrome c_2 and antiserum to RHP suggesting that cytochrome c_2 and RHP might be structurally similar. However, the possibility of a small quantity of RHP contaminating the crystalline cytochrome c_2 has not been ruled out as a cause for this cross-reaction. Results of these studies strongly uphold the conclusion that RHP is not an artifact produced by purification procedures.

17 *Biochemistry*

THE ISOMERIZATION REACTION IN HUMAN MERCAPTALBUMIN. Patricia Clark*, Michael R. Rachinsky*, and Joseph F. Foster. Dept. of Chemistry, Purdue University, Lafayette, Indiana.

The authors have conducted an extensive study of the behavior of human mercaptalbumin in moving boundary electrophoresis in the low-pH range at two ionic strengths, 0.02 (0.2% protein) and 0.10 (0.4% protein). In both cases chloride was the sole counterion. The results are in excellent agreement with those previously published on bovine albumin (Aoki and Foster, J. Am. Chem. Soc. 78: 3538, 1956; 79: 3385, 3393, 1957; 80: 1117, 5215, 1958). Two moving boundaries were invariably seen in the pH range 3.5-4.5, the relative composition changing with pH in accord with the equilibrium $N + 3\ H^+ \rightarrow F$. A relatively high degree of enantiography was observed throughout this pH range. The difference in mobilities between N and F boundaries was compatible with the uptake of three protons and no change in chloride binding. An improved interpretation of the anomalous titration behavior of the protein in the carboxyl region has been attempted utilizing quantitative mobility data to evaluate the electrostatic interaction parameter. Jirgensons (Arch. Biochem. Biophys. 78: 227, 1958) demonstrated differing behavior in optical rotation between bovine and human albumins in the pH range 3.5-4.0. This difference must be due to other factors than the failure of the human protein to undergo isomerization, as was also suggested by Jirgensons.

18 *Biochemistry*

ELECTROPHORESIS OF CALF THYMUS HISTONES. Claude McClure* and J. Logan Irvin. Univ. of North Carolina, School of Medicine, Chapel Hill, N. C.

Calf thymus deoxypentose nucleoprotein (DPN) was isolated by a modification of the method of Mirsky and Pollister. After purification of DPN by repeated solution in 1M NaCl and precipitation at ionic strength 0.15, histones were extracted from DPN by two procedures: (a) 0.2N HCl, followed by dialysis and lyophilization, or (b) the barium acetate extraction described by Crampton, Moore, and Stein. Electrophoresis of the histones was studied by the moving boundary method in a Spinco apparatus, model H, at various values of pH, ionic strength, and total protein concentration. Enantiography of electrophoretic patterns was obtained only at total protein concentrations of 0.5 per cent, or less. At pH 4.5 and ionic strength 0.1, two components were observed with descending mobilities of 8.8 and $6.7 \times 10^{-5}\ cm^2 \times s^{-1} \times v^{-1}$. At pH 5.6, ionic str. 0.1, three components were observed with mobilities of 8.4, 7.4, and $6.4 \times 10^{-5}\ cm^2 \times s^{-1} \times v^{-1}$. Three components also were observed at pH 7.3 and 9.3, and additional minor components were found in some preparations in which dialysis was avoided. Lack of enantiography of patterns and other anomalies were observed at total protein concentrations of 1-2 per cent, particularly at low ionic strength. Resolution of components improved above pH 7, but with some interaction.

19 *Biochemistry*

FRACTIONATION AND CHROMATOGRAPHIC ANALYSIS OF ANIONIC NUCLEAR PROTEINS. Samir M. Amer* and Harris Busch. Dept. of Pharmacol., Univ. of Illinois, Coll. of Med., Chicago, Ill.

The biosynthesis of anionic nuclear proteins was found to be markedly inhibited by 5-bis(2-chloroethyl) aminouracil (Busch, Amer, and Nyhan, J. Pharmacol. 127: 195, 1959). To fractionate these proteins, successive extraction with increasing concentrations of NaCl (0-5 M) was employed, one hr. following I.P. injection of 10 ucuries of L-arginine-U-C^{14}. The total extract contained 74% and 67% of the dry weight of the nuclei of tumor and liver respectively and 80% and 63% of the radioactivity respectively. Proteins with the highest S.A. in the tumor were extracted with water, while those of liver were extracted with 0.5 M NaCl. Following extraction of original nuclear preparations with 0.14 M NaCl, the proteins were chromatographed on DEAE-cellulose columns with 0.1 M NH_4 acetate as the eluting agent. In both tumor and liver, 4 major protein peaks were eluted. Chromatograms of tumor contained a total of 21 protein peaks and 29 radioactive peaks while chromatograms of liver contained a total of 13 protein peaks and 23 radioactive peaks. The most radioactive peaks in the tumor were radioactive peaks 3 and 7, while the most radioactive peaks in the liver were peaks 8, 10 and 16.

20 *Biochemistry*

USE OF MICROPOROUS MEMBRANES IN FORCED-FLOW ELECTROPHORESIS. M. Bier, Priscilla Taitelbaum*, Nurettin Denis* and I. Bier*. Dept. of Protein and Enzyme Chemistry, Institute of Applied Biology, New York, N.Y.

While Selective Plasmapheresis (Federation Proc. 18,756, 1959) allows the separation of gamma globulins from whole blood of man, sheep or dog, the erythrocytes of rabbits have a too low electrophoretic mobility to permit separation. Microporous membranes physically prevent the passage of the erythrocytes into the gamma globulin fraction, while the fractionation of soluble plasma components is carried out as usual. Microweb filters (Millipore Corp.) and microporous vinyl membranes (ESB-Reeves Corp) were employed, their pore-size ranging from a fraction to several microns.

The application of the electric field in the forced-flow apparatus prevents clogging of the membranes, which takes place very rapidly in the absence of current. The same principle was applied to the filtration and concentration of suspensions of yeast and bacteria. It was found that the application of the electric field greatly increases not only the capacity of the filter but also its selectivity; the process is thus useful for the preparation of bacteria-free filtrates, and is presently under investigation for water treatment. (Supported by the Whitehall Foundation, Inc.)

21 *Biochemistry*

PHYSICOCHEMICAL PROPERTIES OF CREATINE KINASE. William A. DaCosta*, James R. Sparks* and Felix Friedberg. Department of Biochemistry, College of Medicine, Howard Univ., Washington, D.C.

Some physicochemical properties of crystalline creatine kinase prepared according to the method of S.A. Kuby et al. (J. Biol. Chem. 209, 191, 1954) have been studied. Sedimentation in the ultracentrifuge, viscosity and ultraviolet spectra of this protein have been examined at various pH's. These data have been correlated with information obtained from titration curves. In all these properties changes occur at a pH below 5 and also above 9.5, i.e. the sedimentation constant falls, viscosity rises, the ultraviolet absorption maximum on the alkaline side is shifted and irregularities in the titration curve appear.

22 *Biochemistry*

POSSIBLE SYNTHETIC TERTIARY BONDS IN PROTEINS. Finn Wold (intr. by H. E. Carter) Division of Biochemistry, University of Illinois, Urbana.

The reaction of bovine serum albumin (BSA) and ribonuclease (RNAse) with p,p'-difluoro-m,m'-dinitrodiphenylsulfone (FNPS) has been studied in an attempt to introduce new tertiary bonds into these proteins. BSA reacts readily with 10 moles of FNPS per mole of protein (1% protein solution in carbonate buffer, pH 10.7). The sedimentation, viscosity and rotation of the product (NPS-BSA) are not significantly different from those of the native protein. Spectrophotometric evidence shows that both ends of FNPS have reacted, but there is no indication that dimer formation occurs under the conditions used. NPS-BSA and BSA were subjected to denaturing agents such as heat, acid, base, urea, urea and thioglycolic acid, and performic acid oxidation, and were subsequently compared in the ultracentrifuge. In all cases the sedimentation data show that NPS-BSA is more resistant to the denaturing agents than is BSA itself. RNAse also reacts readily with FNPS, and retains full activity after reaction with 4-5 moles of FNPS per mole of protein. Thus, FNPS may react with two sites in the polypeptide chain, thereby introducing new stabilizing "tertiary" bonds into the protein molecule, apparently without changing its native configuration. Electrophoresis indicates that the epsilon-amino groups of lysine are involved in the reaction.

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23 *Biochemistry*

INVOLVEMENT OF A TRYPTOPHAN RESIDUE IN THE REACTION OF DIISOPROPYL PHOSPHOFLUORIDATE (DFP) WITH α-CHYMOTRYPSIN. George P. Hess and John F. Wootton*, Dept. of Biochemistry, Cornell Univ., Ithaca, N. Y.

We have previously reported that the catalytic hydrolysis of p-nitrophenyl acetate by α-chymotrypsin results in an monoacetyl α-chymotrypsin intermediate which can isomerize. (J. Am. Chem. Soc. 81, 2594 (1959); Nature 184, 113 (1959)) The ultraviolet difference spectrum between monoacetyl α-chymotrypsin and chymotrypsin is closely similar to that between DIP-α-chymotrypsin and α-chymotrypsin. (Molar absorption difference coefficients (1 cm. cell) are: 290 mμ: + 720; 283 mμ: + 280.) These difference spectra disappear on deacylation of the monoacetyl enzyme. The difference spectra of DIP-α-chymotrypsin persist over the pH range 1-13, in aqueous solution and after denaturation in 8 M urea for 48 hours at 25°C. A colorimetric determination using the phenol reagent reveals 10 tyrosine and tryptophan residues in DIP-α-chymotrypsin but 11 such residues in α-chymotrypsin. Spectrophotometric titration of tyrosine residues in water and in 8 M urea do not reveal differences between DIP-α-chymotrypsin and α-chymotrypsin. The data are consistent with a modification of a tryptophan residue in DIP-α-chymotrypsin and presumably in monoacetyl-α-chymotrypsin. Attempts to locate this tryptophan residue are in progress. (Supported in part by N.S.F.)

24 *Biochemistry*

ELECTROPHORETIC SEPARATION OF SERUM PROTEIN ON CYANOGUM GEL Samuel Raymond and D. Senesky (intr. by J.G. Reinhold)

Cyanogum is a synthetic derivative of acrylamide which can be dissolved in buffer solutions of any pH. In suitable closed molds the solutions can be cast into uniform, thin and reproducible sheets of gel for use as an electrophoresis medium. The gel is entirely non-carbohydrate in nature. Because of the high water content of cyanogum gels, a completely closed electrophoretic apparatus with direct-contact cooling of the gel surface must be used. Specimens can be applied in a slit on horizontal gel layers, or, in vertical gel apparatus found advantageous in present studies, by layering the protein solution over the upper edge of the gel sheet. Staining with amidoblack is complete within five minutes. The excess dye is removed quantitatively in a circulating bath of special design which continuously passes the destaining solvent thru a decolorizing charcoal bed, leaving a blue pattern on a colorless transparent background. The serum protein patterns obtained on Cyanogum gel include numerous subcomponents and are qualitatively similar to those obtained on starch gel. The similarity in behavior of two chemically different gels suggests that pore size rather than chemical structure of the gel is the important factor in determining the number of components and degree of resolution obtained.

25 *Pharmacology*

TRANSFORMATION OF ALPHA CRYSTALLIN AT ALKALINE pH. R. A. Resnik and J. Papaconstantinou (intr. by E. Trams.) Natl. Insts. of Health, Bethesda, Md. and Carnegie Inst. of Wash., Dept. of Embryology, Balt., Md.

Calf lens alpha crystallin undergoes a transformation at acid and neutral pH which is a function of ionic strength. Studies of the effect of low ionic strength at alkaline pH have revealed an alteration of the molecule which is not a function of this parameter. In the ultracentrifuge a single boundary is obtained at pH 7 - 8 in 0.02 M NaCl and at pH 7 in 0.005 M PO_4. At pH 8 in 0.005 M PO_4 about 25% of the protein is transformed to material with a lower S_{20}. The addition of NaCl to the protein in phosphate at pH 8 does not alter the pattern obtained in phosphate at this pH. The addition of phosphate to the protein in NaCl leads to the appearance of transformed material. If EDTA is added to the protein in phosphate at pH 7 and the pH then adjusted to 8, no transformed material appears. Cysteine reduces the rate of the transformation but does not reverse the process. Bisulfite, added to a solution of protein in which about half the material has been altered, enhances the appearance of slower sedimenting material. Monovalent mercury does not prevent the transformation from taking place. It is concluded that at alkaline pH, in the presence of phosphate, trace metals present in the reagents cause a splitting of disulfide bonds.

26 *Immunology*

PROPERTIES OF A CADMIUM PROTEIN FROM EQUINE KIDNEY CORTEX. Jeremias H. R. Kägi (intr. by George P. Berry) Biophysics Research Laboratory of the Dept. of Med., Harvard Medical School and Peter Bent Brigham Hospital, Boston, Mass.

The recognition of the specific association of a metal with a biological macromolecule is an important first step in understanding its function. Cadmium, with no known biological function, occurs in parenchymatous organs of many species. A cadmium protein from horse kidney cortex (Margoshes and Vallee, J.A.C.S. 79: 4813, 1957) has been purified further, to contain 50,000 μg of cadmium and 11,000 μg of zinc per gram dry weight, corresponding to 8 gram atoms of metal per mole (molecular weight 13,000) and accounting for virtually all of the cadmium and 60% of the zinc in this organ. The characteristics of the protein are unusual; 13% N; no aromatic amino acids (i.e. no absorption maximum at 280 mμ); 7% sulfur, accounted for by an exceptionally high cysteine content. A characteristic spectral absorption at 250 mμ may reflect the last circumstance. Cadmium and zinc are removed differentially as a function of pH. The degree of accumulation of these elements in one protein is highly unusual in biological systems. The predilection of cadmium and zinc for sulfur bonding, and their capacity for isomorphically replacing each other, may be important considerations in the physical and biological chemistry of this unique molecule.

217. PROTEINS, POLYPEPTIDES—Structure

1 *Physiology*

ELECTROLYTIC REDUCTION OF DISULFIDE BONDS OF INSULIN. Gabor Markus (intr. by Julian L. Ambrus). Roswell Park Memorial Institute, Buffalo 3, N.Y.

The disulfide bonds of insulin in 1% solution, bicarbonate-carbonate buffer, pH 8.5 are reduced using an electrolytic method, at 10 volts and 20 m Amps. The course of reduction is followed by amperometric titration of the sulfhydryl groups. The reduction of the first two disulfides proceeds at a significantly higher rate than that of the last one. Measurements of optical rotation show a steep rise in levorotation accompanying the reduction of the first two disulfides (-40° to -69°). Reduction of the last disulfide reverses this trend bringing down the rotation to -64°. The reduction appears to be specific for the disulfide bonds, no change in the ultraviolet spectrum having been observed. No hydrolysis of peptide linkages could be demonstrated. The identification of the last disulfide bond to be reduced is now in progress using paper electrophoresis at pH 5.5 in 8 M urea. Preliminary results indicate that this bond may be predominantly the intrachain disulfide of the A chain. Structural implications of these findings will be discussed. (Supported by grant A-2832 from the U.S. Public Health Service.)

2 *Physiology*

AMINO ACID COMPOSITION OF HUMAN GAMMA GLOBULIN SUBFRACTIONS. Kenneth R. Woods* and Ralph L. Engle, Jr. New York Hospital - Cornell Medical Center, New York City.

The gamma globulin fraction of serum proteins is comprised of a spectrum of proteins having different physicochemical and immunochemical properties. To examine how covalent structure may relate to these diverse biochemical properties, Fr. II (Pentex Lot #6409) was first electrophoretically distributed throughout a central zone of a powdered PVC block, diethylbarbiturate buffer, pH8.6, $\mu/2$ 0.050. The resulting continuous γ-globulin zone was sliced into eight 1 cm. segments and the protein contained in each was separately eluted, dialyzed, lyophyllized and weighed. Zone electrophoresis of each subfraction in starch gel revealed that distinctly different electrophoretic components had been obtained. Hydrolysates (6N-HCl, 20 hr. 110°C.) were analyzed for amino acids by recording ion exchange chromatography. Each fraction had a significantly different though similar amino acid content. There was a general gradation in amino acids carrying free ionic groups in the protein molecules with increasing electrophoretic mobilities. Arg., hist., and lys. were lower in the more electronegative fractions, and asp. and glu. were higher. It is concluded that human γ-globulins can be electrophoretically fractionated into subfractions detectably different in amino acid composition. (USPHS Grant C1905)

3 *Biochemistry*

α- AND β-MSH IN MONKEY PITUITARY GLANDS. Teh H. Lee*, Aaron B. Lerner and Vina Buettner-Janusch*. Yale Univ. School of Med., New Haven, Conn.

The failure to demonstrate the presence of α-MSH (melanocyte stimulating hormone) in material from human pituitary glands prompted us to search for such a substance in pituitary glands of other primates. An oxycellulose purified concentrate prepared from fresh, frozen monkey pituitary glands was fractionated on a carboxymethyl cellulose column using ammonium acetate as eluent. α- and β-MSH as well as ACTH emerged as separate components. Preliminary studies on their physicochemical properties indicated that these hormones probably possess amino acid sequences closely related to their counterparts in pituitary glands of hogs, cattle and sheep.

4 *Biochemistry*

S-CARBOXYMETHYL PAPAIN. J. R. Kimmel and A. J. Parcells*, Univ. of Utah Coll. of Med., Salt Lake City, Utah.

Reduction of denatured papain with $NaBH_4$ followed by alkylation with iodoacetate, quantitatively converts all cysteine residues to S-carboxymethyl cysteine. Like native papain, S-carboxymethyl papain contains 8 moles of sulfur per mole of protein but only 6 residues of S-carboxymethyl cysteine. Methionine is absent and no other sulfur containing component is evident. Analysis shows that tryptophan has not been altered and that essentially no alkylation of lysine has occurred. Some loss of tyrosine has apparently occurred. A number of new amino end groups appear as a result of $NaBH_4$ reduction. DNP-isoleucine is present in stoichiometric amounts but the others are present in less than stoichiometric quantities. The appearance of new N-termini is not associated with the appearance of new C-termini demonstrable with carboxypeptidase. Apparently, reductive cleavage of peptide bonds has occurred. Sequence studies with S-carboxymethyl papain have permitted isolation of the following peptides containing tryptophan from tryptic digests:

....Ileu.Pro.Glu.Tyr.Val.Asp.Try.Arg....

....(Asp,Thr,Ser,Glu,Gly_3,Tyr,Try_2)Ileu.Arg....

5 *Biochemistry*

HISTIDINE AND PHENYLALANINE SEQUENCES IN PAPAIN. Albert Light* and Emil L. Smith. Univ. of Utah College of Med., Salt Lake City, Utah.

The two histidine residues of oxidized papain have been isolated in peptides after chymotryptic digestion. One of these is present in Gly.Ileu.His.Tyr. The other was found in a heptadecapeptide which was shown to be part of the following 38 residue sequence:

....Arg.Gly.Gly.Ileu.Phe.Val.Gly.Pro.$CySO_3H$.Gly.$AspNH_2$.Lys.Val.$AspNH_2$.Ala.Val(His,Ala)Val.Gly.Ala.Tyr.(Asp_2.Ser,Glu_2,$CySO_3$,Arg,Leu)(Asp,Ser,Glu,Arg,Try,Gly,Leu,Tyr)....

In addition to the phenylalanine residue in the above sequence, the other three of papain have been found in the following sequences:

....Ala.Ser.Phe....

....Thr.Ser.Ser.Phe....

....Glu(Gly,Ala_2)Lys.Asp.Phe....

The last two peptides overlap with known peptides from tryptic digests. These and other sequences for the partial structure of papain will be presented.

6 *Biochemistry*

REDUCTION OF DISULFIDE BONDS IN PROTEINS BY SODIUM BOROHYDRIDE. Arthur M. Crestfield*, Janusz Skupin*, Stanford Moore, and William H. Stein, Rockefeller Institute, New York City.

When the reduction (cf. Moore, S., Cole, R. D., Gundlach, H. G., and Stein, W. H., Symp. on Proteins, 4th Internat. Congress Biochem., Vienna, 1958) is performed in the presence of 0.02 o/o Versene, any tendency for reductive cleavage of peptide bonds is minimized. The combined use of Versene and a nitrogen barrier makes it possible to remove excess borohydride by bringing the reaction mixture to pH 7 in the presence of Dow Corning Antifoam B, without reoxidation of the -SH groups. These can then be alkylated by iodoacetate in 2 mins. at pH 8.6 without detectable side reactions with methionine or lysine residues. The resulting derivatives can usually be separated from the reagents by dialysis, preceded if necessary by rapid removal of excess iodoacetate on Dowex-1 acetate. By-products of the borohydride reaction cause 10-35 o/o loss of methionine during acid hydrolysis, unless the modified protein is first adsorbed on the acid form of IRC-50 and eluted by 50 o/o acetic acid. When the protein contains more than one peptide chain, this step may result in fractionation, as it does in the case of insulin. The method has been used successfully for the isolation of reduced-carboxymethylated ribonuclease, β-lactoglobulin, and lysozyme.

7 *Biochemistry*

INHIBITION OF AMYLASES BY BERYLLIUM SALTS. Robert L. McGeachin. Dept. of Biochemistry, Univ. of Louisville School of Medicine, Louisville, Ky.

Beryllium salts inhibit hog, dog, rat and human amylases to varying degrees. The addition of magnesium, barium, and strontium salts do not inhibit amylase in our experiments. Beryllium sulfate and chloride are more effective than the acetate and the addition of other acetate salts reduces the inhibiting effect of beryllium sulfate and chloride. Hog pancreatic amylase is inhibited 85-95% by 10^{-3} M $BeSO_4$, whereas in the presence of 10^{-3} M acetate the inhibition dropped to 40%. Inhibition by 10^{-3} M Be acetate is 30-35%. Human serum amylase is inhibited 10-20% by 10^{-3} $BeSO_4$, human salivary amylase 70%, but if bovine plasma albumin in an amount equivalent to the protein in serum is added to the saliva, the inhibition of the salivary amylase is only 10-20%. The presence of gelatin also modifies the inhibiting effect of beryllium salts. It is postulated that beryllium salts produce their effects on the amylases either by replacing the calcium which all amylases are known to contain or by combining with the free carboxyl groups in such fashion that the structure of the original molecule is deformed and the enzyme activity reduced.

8 *Biochemistry*

ISOLATION AND PARTIAL CHARACTERIZATION OF INSOLUBLE PEPTIDE FROM TRYPTIC DIGESTS OF TOBACCO MOSAIC VIRUS (TMV) PROTEIN. Akira Tsugita and L. K. Ramachandran (intr. by W. M. Stanley) Virus Laboratory, Univ. of Calif., Berkeley.

The high molecular weight (4200) peptide (I-peptide) isolated from tryptic digests of TMV protein by isoelectric precipitation was purified by repeated precipitation at pH 4.5 and by 30% Na_2SO_4, and finally by weak anion exchange chromatography. The amino acid composition was determined by column techniques. The peptide consisted of about 40 amino acids (Cys_1, Asp_4, Thr_4, Ser_{4-5}, Glu_5, Pro_2, Gly_1, Ala_4, Val_1, $Ileu_{3-4}$, Leu_5, Tyr_1, Phe_{2-3}, Try_1, $(NH_2)_7$ including only one basic amino acid, arginine). The N-terminal sequence was shown to be that of the intact protein chain, namely, N-acetyl-Ser-Tyr. The C-terminal sequence of the peptide was established by hydrazinolysis and carboxypeptidase digestion, as well as by isolation after chymotryptic digestion as (Thr, Ala, Glu $(NH_2)_2$, Arg) and (Ala, Glu (NH_2), Arg). From the chymotryptic hydrolysate an isoelectrically insoluble peptide was also isolated and partially characterized by enzymatic and chemical methods.

XUM

9 *Biochemistry*

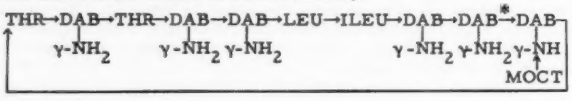
SEPARATION AND AMINO ACID ANALYSIS OF THE ALPHA AND BETA COMPONENTS OF COLLAGEN. Karl A. Piez and Ellinor Weiss.* National Institutes of Health, Bethesda 14, Md.

Denaturation of collagen under mild conditions is known to result in the formation of two components, designated alpha and beta, which sediment at different rates in the ultracentrifuge. We have examined the chromatographic behaviour on carboxymethyl cellulose of calf skin collagen, denatured by mild heating in acid solution. A partial separation of the two components was obtained. Appropriate fractions were rechromatographed and the purified components were examined in the ultracentrifuge and analyzed for their amino acids. They were found to be essentially pure. Their amino acid compositions were similar but differed significantly for many of the amino acids. Glutamic acid, aspartic acid, proline and alanine differed by nearly 10%. Valine, isoleucine, leucine, hydroxylysine, lysine, and histidine were 30 to over 100% higher in one than in the other. Analysis of the unfractionated collagen indicated that it was composed of approximately equal amounts of the two components.

10 *Biochemistry*

THE STRUCTURE OF CIRCULIN B. Henry Koffler and Toshio Kobayashi. Dept. Biol. Sci., Purdue U., West Lafayette, Ind.

Cells of Bacillus circulans produce several lipopeptide antibiotics, the circulins, of which A and B are the main products. Both are remarkably active against many gram-negative bacteria and contain L-α-γ-diaminobutyric acid (DAB), L-threonine, D-leucine, L-isoleucine, and (+)6-methyloctanoic acid (MOCT) in the molar relationship of 6:2:1:1:1. The structure of circulin A has been described [Absts. Communs. 4th Intern. Cong. Biochem., 9(1958); Science, 130, 1419 (1959)]. Interestingly, circulin B consists of the same cyclic peptide as does circulin A, and differs from the latter only in the location of MOCT. This was determined from the following fragments of the molecule obtained by partial hydrolysis: THR→DAB→THR; DAB→THR→DAB→DAB→LEU; LEU-ILEU→DAB→DAB; and DAB→DAB(γ-NH-MOCT)→THR. The structure of circulin B is as follows (the asterisk indicates the attachment of MOCT in circulin A):



11 *Biochemistry*

SOME OBSERVATIONS ON HUMAN PLASMA MERCAPTALBUMIN AND NON-MERCAPTALBUMIN. T. P. King (intr. by E. H. Ahrens.) Rockefeller Institute, New York.

Dissolution of human plasma albumin in a solvent system of ethanol, n-propanol, ammonium sulfate and water was found to result in dimerization of a portion of the albumin. Countercurrent distribution in this system separated the mixture into two bands, the dimer ($S_{20,w}$=6.23) with K of 0.20 and the monomer ($S_{20,w}$=4.30) with K of 0.57. Both fractions were devoid of titratable sulfhydryl group. Further experiments have indicated that the dimer is a double molecule of two mercaptalbumin molecules linked by a disulfide bond. Treatment of the dimer or monomer fraction with thioglycolate at pH 5 gave a monomeric product with similar properties in both cases. The products contained titratable sulfhydryl group, yet they showed only little tendencies to dimerize in the described system. The results suggested that an intramolecular disulfide exchange had taken place. Upon digestion by carboxypeptidase A, the dimer and the monomer fractions showed similar rates of release of amino acids. Their probable C-terminal sequence is...(Val,Leu,Ser,$GluNH_2$)·Gly·Ala·Leu. The two fractions were found to have the same amino acid compositions. The results suggest that the mercaptalbumin and non-mercaptalbumin are probably proteins with identical primary sequence but different in their tertiary structures.

12 *Biochemistry*

THE SEPARATION OF THE TRYPTIC PEPTIDES FROM THE ALPHA CHAIN OF HUMAN HEMOGLOBIN A BY COUNTERCURRENT DISTRIBUTION. W. H. Konigsberg and R. J. Hill (intr. by D. W. Woolley.) Rockefeller Institute, New York.

In studies aimed at elucidating the amino acid sequences of human hemoglobin A, we have employed methods that enabled us to separate large quantities of peptides obtained from tryptic hydrolysis of the alpha chain (N-terminal Val·Leu). The two chains were separated by C.C.D. (Hill, R. J. and Craig, L. C., J. Am. Chem. Soc., 81, 2272 (1959)). The alpha chain was digested at pH 9.0 for 8 hrs. with trypsin prepared from chromatographically purified trypsinogen. The tryptic peptides were cleanly separated into two fractions by dialysis through 18/32 Visking cellophane with 0.2 M acetic acid as a solvent. The dialyzable peptides (80% of the total by weight) were distributed in a phenol, ethanol, aqueous HCl system. The peptide bands were recovered and redistributed in a 2-butanol, acetic acid, trifluoroacetic acid system. The non-dialyzable portion of the tryptic digest was distributed in a 2-butanol, acetic acid, dichloroacetic acid system to obtain the "core" peptide. The purity of the peptides was ascertained by paper chromatography and paper electrophoresis. Using these methods, it has been possible to isolate peptides from gram quantities of each chain and at the same time to determine the yield and quantitative amino acid composition of each peptide.

13 *Biochemistry*

INACTIVATION OF CARBONIC ANHYDRASE BY PROTEOLYTIC ENZYMES. James B. Gilbert. Clayton Foundn. Biochem. Inst., Univ. Tex., Austin.

Addition of either chymotrypsin, papain or ficin, plus required activators, to a lyophilized carbonic anhydrase preparation dissolved in a buffer at a pH favorable to proteolysis, together with incubation at 37° resulted in the disappearance of carbonic anhydrase activity from the digest. Certain proteolytic enzymes e.g. trypsin, had no such effect. Furthermore, under comparable conditions, chymotrypsin and papain had no appreciable action on the enzymatic activity of uricase, a copper metallo-enzyme. The carbonic anhydrase preparation employed represented the fraction which remained soluble after the chloroform-ethanol denaturation of the hemoglobin in a beef erythrocyte lysate. Carbonic anhydrase activity was measured by either of two "changing-pH" methods and in one instance by manometric means. One possible interpretation of these findings is that there was direct proteolytic attack on this zinc-containing enzyme. Also accomplished was the separation of carbonic anhydrase from hemoglobin using carboxymethylcellulose as adsorbent; additionally this enzyme was adsorbed on and eluted from diethylaminoethylcellulose. (Supported by an Established Investigatorship, American Heart Assoc., and NIH grant RG-6299.)

14 *Biochemistry*

EVIDENCE FOR THE INTRAMOLECULAR STABILIZATION OF THE COLLAGEN FOLD. William F. Harrington and Peter H. von Hippel, Natl. Heart Inst., Bethesda, Md., and Dept. of Biochemistry, Dartmouth Univ., Hanover, N.H.

In an earlier communication (von Hippel and Harrington, Biochim. et Biophys. Acta, in press) it has been suggested that the gelatin→collagen fold transition involves three distinct steps. In this report we wish to present additional evidence that the second step of the process, the formation of the poly-L-proline II type helix, is stabilized intramolecularly. The kinetics of formation of the collagen fold configuration at low temperature have been followed at very low gelatin (ichthyocol) concentrations through measurement of the optical rotatory changes at a wave length of 265 mμ. The rate of change of specific rotation follows first-order kinetics at all concentrations examined (between 1.5 and 0.09 mg/ml) but the velocity constants at a given temperature are independent of the protein concentration. Moreover the final value of specific rotation characteristic of the collagen fold is identical at all concentrations. Thus the configuration responsible for rotation is formed intramolecularly and does not require stabilization through interaction with other gelatin chains. No formation of the helix is detected in the presence of 8 M urea at low temperatures suggesting that the intramolecular stabilization of the helix occurs through hydrogen bonding.

15 *Biochemistry*

EPSILON AMINO GROUP OF LYSINE, A BRANCH POINT IN COLLAGEN STRUCTURE. Milton Levy, Louis Fishman* and Guillermo Cabrera* N.Y.U. College of Dentistry, N. Y.

Achilles tendon collagen was swollen in 0.01N HCl for a day at 5°C and then treated with nitrous acid or nitrosyl chloride. The excess was removed with sulfamic acid, the mixture was dried and hydrolysed in 6N HCl for 18-24 hrs. The total of acid and neutral amino acids and the separated basic amino acids were determined after separation by chromatography. Nitrous acid treatment destroyed all hydroxylysine and lysine as well as 70-80% of the neutral and acid fraction. This indicates that some hydrolysable form of nitrous acid survived the sulfamic acid and reacted with the amino acid liberated by hydrolysis. This experiment therefore cannot exclude the existence of protected epsilon amino groups in the collagen. Nitrosyl chloride treatment left all the neutral-acid fraction and 35 ± 3% of the lysine. It destroyed all the hydroxylysine. In ichthyocol 40 ± 3% of the lysine and none of the hydroxylysine survived. Ribonuclease loses all of its lysine. Treatment with nitrosylchloride indicates that several peptides separated by chromatography from tryptic hydrolysates also contain protected lysine. Our experiments indicate that a third of the lysine in collagen does not have free epsilon amino groups. (Supported by grants from N.I.H. and N.S.F.)

16 *Biochemistry*

AMINO ACID SEQUENCE OF CYTOCHROME c, E. Margoliash (intr. by N. Weissman). Univ. of Utah Coll. Med., Salt Lake City, Utah.

The composition of horse-heart cytochrome c reinvestigated in both acid and enzymic hydrolysates is:
$Ala_6, Arg_2, Asp_8, 1/2Cys_2, Glu_{12}, Gly_{11}, His_3, Ileu_6, Leu_7, Lys_{18}, Met_2, Phe_4, Pro_5, Ser_2, Thr_9, Try, Tyr_4$.
The following (and other) peptides have been obtained in pure form from chymotryptic digests by ion-exchange chromatography, partition chromatography and electrophoresis:
(heme containing peptide: $Lys_5, His_2, Arg, Asp_2, Thr_3, Ser_2, Glu_6$, Pro, Gly_5, Ala_3, Val_2, Ileu, Leu, 1/2Cys$_2$); (Lys_3, Asp, Glu, Gly_2, Val, Ileu, Phe); (Lys, His, Asp, Thr, Pro, Gly, Leu); (Lys_2, Asp, Thr, Glu, Ala); (Lys_2, Asp, Glu, Pro, Leu, Tyr); (Lys, Thr, Pro, Gly, Met, Ileu); (Lys, Thr, Glu_2, Met, Leu); (Lys, Try, Thr, Gly, Ileu); (Lys, Arg, Thr, Glu, Pro, Gly_3, Ala, Phe); (Lys_2, Arg, Asp, Thr, Glu_2, Gly, Ala, Ileu, Leu, Tyr); (Lys, His, Asp, Thr, Pro, Gly_2, Leu_2, Phe); (Lys, Thr, Glu_2, Leu); (Lys, Asp_3, Thr, Ala); (Lys_2, Gly, Ala, Ileu); (Gly, Leu, Tyr); (Ileu, Phe); (Glu, Tyr); etc.
The composition of the isolated peptides comprises more than the protein composition, thus indicating the presence of overlapping sequences. The relation of these peptides to the overall sequence of the protein will be discussed.

17 *Biochemistry*

SEQUENCE STUDIES OF PEPTIDES FROM AN ENZYMATIC DIGEST OF COLLAGEN. Ralph B. Arlinghaus* and Milan A. Logan. Dept. of Biological Chemistry, Univ. of Cincinnati College of Medicine, Cincinnati, Ohio.

The sequence alanyl-hydroxyproline appears in two peptides isolated from cattle Achilles tendon collagen. One, a tripeptide (Gly.Ala.Hypro.) and, the other, a dipeptide (alanylhydroxyproline) account for 6.2 per cent of the hydroxyproline content of collagen. Collagen was digested by the exoenzymes of Cl. histolyticum. The digest was repeatedly fractionated over columns of Amberlite IRC-50 in the pyridine cycle or hydrogen cycle with water as the eluent and on columns of Al_2O_3 with 76 per cent ethanol ammonia eluent. The N-terminal sequence was determined by the Edman phenylthiohydantoin procedure. Two additional peptides have been isolated on columns of IRC-50, Al_2O_3 and Dowex 50-X4. The amino acid composition of one of the peptides is glycine, glutamic, alanine, proline and of the other peptide glycine, glutamic, and proline. The N-terminal sequences glycyl-glutamyl- and glycyl-prolyl- have been found in these two peptides. Work on complete sequence is now in progress.

18 *Biochemistry*

STUDIES OF THE COENZYME BINDING SITES OF DPN-LINKED DEHYDROGENASES. R. H. McKay*, S. Shifrin* and N. O. Kaplan. Grad. Dept. of Biochemistry, Brandeis Univ., Waltham, Mass.

The interaction of DPN analogues with dehydrogenases has prompted an investigation of the nature of the coenzyme binding sites of beef heart lactic dehydrogenase (LDH), and other DPN-linked dehydrogenases. The acetylpyridine analogue of DPNH (APDPNH) has been found to bind strongly to beef heart LDH, and to protect it from proteolysis by a bacterial protease from Bacillus subtilis. The oxidized acetylpyridine analogue (APDPN) also shows some protective effect. DPN and DPNH have little influence on the proteolysis. The cleavage of bovine serum albumin and ribonuclease are not protected by the coenzymes. Binding of APDPNH also considerably retards the loss of enzymatic activity of LDH resulting from the proteolysis. Utilizing fluorescence measurements, it has been found that most of the APDPNH remains bound after extensive proteolysis, which is sufficient to destroy all enzymatic activity. The reduced analogue appears to be bound to a peptide resulting from the proteolysis. In the course of these studies it was noted that a change in the characteristic protein fluorescence of the beef heart LDH as well as with bovine serum albumin occurs after digestion with the bacterial protease. This change can be followed by a new fluorescent emission at 395 mμ which has a maximum excitation at 315 mμ.

19 *Biochemistry*

AN AMINO ACID DIFFERENCE BETWEEN HUMAN HEMOGLOBINS A AND A_2. A.O.W. Stretton and V.M. Ingram (Intr. by G.M. Brown). Div. of Biochemistry, Mass. Instit. of Technology, Cambridge 39, Mass.

Hemoglobin A_2 is a normal, though minor, component found in the hemolysates of adult humans. We wished to establish its relationship to the chemical structure of the normal major hemoglobin (A). Hemoglobin A_2 has been prepared by large-scale electrophoresis on a starch block, and purified on CM-cellulose. After tryptic hydrolysis, the peptide mixture was compared with hemoglobin A tryptic peptides by paper chromatography and paper electrophoresis. It was found that the fastest moving acidic peptide (at pH 6.4) was absent in hemoglobin A_2 digests. Instead we found a new peptide with a different electrophoretic mobility, but with similar quantitative amino acid composition ($val_3 asp_2 gly_3 ala_2$ glu leu arg). However, in the new peptide one of the two glutamic acid residues normally present (hemoglobin A) was replaced by the neutral alanine. On further degradation with elastase and with acetic acid, it appeared that the glutamic acid residue that had been altered was not the same one as that involved in the abnormal human hemoglobin E; a substitution of the other glutamic acid residue in this peptide by lysine is known to occur in hemoglobin E. A few other peptide changes have been observed in tryptic digests of hemoglobin A_2.

20 *Biochemistry*

SOLVENT EFFECTS ON ULTRAVIOLET SPECTRA OF TYROSYL RESIDUES. Michael Laskowski, Jr., Robert H. Cramer*, Theodore T. Herskovits* and Chien-Chyou Wang*. Dept. of Chemistry, Purdue University, Lafayette, Indiana.

Difference spectra of N-acetyl tyrosine ethyl ester and of N-acetyl p-methoxy phenylalanine ethyl ester were studied between various solvents by the double differential (tandem cell) technique. The spectrum of the tyrosyl compound is enhanced by 1) hydrogen bonding 2) raising of the refractive index of the solvent 3) lowering the dielectric constant of the solvent, while the spectrum of the p-methoxy phenylalanyl compound is enhanced only by 2) and 3). The implications of these findings on the "conventional" difference spectra of proteins will be discussed. The experiments further suggest a method for distinguishing between "buried" and "exposed" aromatic groups in proteins. Provided that a small change in the refractive index of the solvent can be made without affecting the protein conformation only the "exposed" groups will be affected. Thus, if any groups are "buried" the resulting spectral shift will be smaller than that obtained in a model experiment. Several preliminary experiments on proteins not containing any tryptophan are in satisfactory agreement with this expectation.

XUM

21 *Biochemistry*

HYPOBROMITE OXIDATION OF AMINO ACIDS AND PEPTIDES. Frederick H. Carpenter and William H. McGregor*. Dept. of Biochemistry, Univ. of Calif., Berkeley.

A study has been made of the oxidation of amino acids and peptides with hypobromite. The studies differ from those of previous workers (Goldschmidt, Wiberg, Nagerl and Martin, Ann. 456:1, 1927; Goldschmidt and Strauss, ibid 471:1, 1929; Friedman and Morgulis, J. Am. Chem. Soc. 58:909, 1936) in that a comparison was made of the course of the reaction in strong alkali vs. buffered solutions at pH 9. Hypobromite oxidation of most of the amino acids yielded the corresponding nitriles of one less carbon atom regardless of the pH. Some amino acids reacted further. At high pH lysine, aspartic and asparagine gave glutaronitrile, di-bromoacetamide and cyanoacetamide, respectively. Oxidation at pH 9 resulted in cyanoacetic acid, glycolonitrile and lactonitrile from aspartic acid, serine and threonine, respectively. Oxidation of peptides led to entirely different reactions at pH 9 and in strong alkali. In strong alkali, a nitrile was obtained from the N-terminal amino acid. Upon acidification of the reaction mixture, the C-terminal amino acid was liberated from a di-peptide, while a molecular rearrangement had taken place with tri- and tetra-peptides. In oxidation at pH 9, di-, tri-, and tetra-peptides were deaminated at the N-terminal amino acid to give α-keto-acyl-peptides. This work was supported by grant A-608 from N.I.H.

22 *Biochemistry*

THE INTERACTION BETWEEN RIBONUCLEASE A AND 2'-CYTIDYLIC ACID. John P. Hummel, Charles A. Nelson* and Dan Ver Ploeg*. Dept. of Biochem., State Univ. of Iowa, Iowa City, Iowa.

Upon mixing approximately equimolar quantities of bovine pancreatic ribonuclease A and 2'-cytidylic acid, the absorbancy at 250-260 mμ is less than the sum of their separate absorbancies; at 290 mμ, on the other hand, the reverse is found. This "hypochromicity" at 260 mμ is greatest at pH 5-6 and at low ionic strengths. It is greater in acetate, imidazole or "tris" than in phosphate buffers. The hypochromicity is also observed to a lesser extent upon mixing ribonuclease A with 3'-cytidylic acid but not with 5'-cytidylic acid or cytidine. From the absorbancy data at pH 7.3 and at an ionic strength of 0.1, the dissociation constant of the complex between ribonuclease A and 2'-cytidylic acid is calculated to be 2.7×10^{-4} M. The same K_I value of the complex was determined enzymatically with the pH-stat using cytidine 2':3'-cyclic phosphate as substrate. (Supported by Grant RG-6071 (C1) of the U.S.P.H.S.)

23 *Biochemistry*

MOLECULAR TRANSFORMATION OF MACROGLOBULINS. H. F. Deutsch and F. C. Greenwood*. Dept. Physiological Chem., Univ. of Wis., Madison, Wis.

Previous studies have demonstrated that Waldenstrom macroglobulins are converted to 160,000 mol. wt. proteins by sulfhydryl reagents and that this transformation is partially reversible. Further examination of this phenomenon has indicated that the extent of reaggregation is a function of the sulfhydryl reagent utilized. Dissociation products formed with alkyl mercaptans show a high degree of reaggregation while those formed by cysteine show little. The reaggregation process is inhibited by alkylating agents in all cases. The units of 160,000 mol. wt. formed by reduction and alkylation of macroglobulins are cleaved to a series of smaller molecules under acid pH conditions at which the native macroglobulins are stabile. This acid catalyzed cleavage is reversed at neutral pH. Sulfhydryl reactions do not appear to be involved in the latter changes since alkylation fails to block the reaggregation. This indicates that two different types of bonds are involved in the molecular transformations under consideration. (Supported by Grant No. C-1786, U. S. Public Health Service.)

24 *Pharmacology*

CROSSLINKING OF COLLAGEN PHOTOCATALYZED BY RIBOFLAVIN. Eloise Kuntz (intr. by W. Van Winkle, Jr.), Ethicon, Inc. Somerville, New Jersey

The formation of thermostable bonds between collagen molecules has been achieved by irradiating collagen in the presence of riboflavin or riboflavin-5-phosphate with visible or near ultraviolet light. Treatment of reconstituted beef tendon collagen results in a lowering of the molecular weight between crosslinks from about 150,000 in control material to an average of 12,000 after treatment. Expressed as thermostable bonds per 360,000 (the molecular weight of tropocollagen), the bonds increase from 2.5 to 30.7.

The crosslinking reaction can be carried out on collagen in solution, in dispersion and in solid form. When a solution or dispersion of collagen is treated there is an increase in viscosity followed by setting to a thermostable gel. The gel exhibits syneresis when heated to about 60°. This corresponds to the familiar heat shrinkage exhibited by solid collagen in water.

The spacial relationship and availability of pairs of reactive sites in the collagen determines the number of bonds which can be formed. The major effect of temperature is through its influence upon the state of the collagen. Crosslinking is low in acid media but rises in alkaline to a maximum at pH 9. Activity is high from pH 9 to 12.

218. PROTEINS, POLYPEPTIDES—Synthesis

1 *Physiology*

THE INCORPORATION OF AMINO ACIDS IN PROTEIN BY CELL-FREE PREPARATIONS FROM MAIZE ENDOSPERM AND GERMINATING SEEDLINGS. Rusty J. Mans and Robert Rabson (intr. by Norman Anderson.) Biology Div., Oak Ridge National Laboratory, Oak Ridge, Tenn.

A system consisting of a 100,000 X g particle and a pH 5.2 precipitate from developing endosperm that catalyzes the incorporation of amino acids into particulate protein has been developed. This system has many characteristics in common with the rat liver microsomal-pH 5 system (P. C. Zamecnik, and E. B. Keller, J. Biol. Chem., 209:337, 1954); it requires ATP or an ATP generating system, is stimulated by GTP and Mg^{++} ions, and is inhibited by treatment with ribonuclease. It is also inhibited by chloramphenicol. Washed particles are inactive but supplementation with the pH 5.2 fraction from maize or rat liver supernatant restores full activity. The latter is about 3 times as active as maize pH 5.2. Activity of the particles depends upon the maturity of the ears at harvest. The incorporating efficiency of this system is 1 to 2% of the added radioactivity. A similar system has been prepared from maize seedlings. Most active preparations are obtained from 2-day-old seedlings and activity diminishes with age. The properties of these two systems will be discussed and compared with the rat liver and pea seedling systems (G. C. Webster, J. Biol. Chem., 229:535, 1959).

2 *Biochemistry*

PROTEIN SYNTHESIS IN REGENERATING LIVER. George A. Braun*, Julian B. Marsh and David L. Drabkin. Dept. Biochem., Grad. School of Med., Univ. of Pennsylvania, Phila.

There is a recognized moderate decrease in plasma albumin after partial hepatectomy. We have found that the rate of catabolism of C^{14}-albumin by slices from regenerating rat liver is unchanged. Net synthesis of plasma albumin at 12, 24, 72 and 144 hours is not significantly different from that in the controls. However, incorporation of C^{14}-amino acids into plasma albumin is significantly altered to the extent of -33, +23, +40 and +75 per cent at the four time periods. Also, though different in pattern of change against time, incorporation into tissue protein is increased by +12, +77, +54 and +16 per cent. The rise in the concentration of free amino acids was +29% (maximal) at 12 hours, diminishing to +2% at 144 hours. On the basis of our data the increased incorporation into albumin cannot be related unequivocally to the degree of biosynthesis. The lowered plasma albumin concentration is attributed tentatively to catabolism by extra-hepatic tissues.

3 *Biochemistry*

STUDIES ON PROTEIN SYNTHESIS WITH RIBONUCLEOPROTEIN PARTICLES FROM PEA SEEDLINGS. I. D. Raacke (intr. by A. D. McLaren). Kaiser Foundation Research Institute, Richmond, California.

It has been reported previously (Raacke, Biochim. Biophys. Acta 34:1, 1959) that unwashed ribonucleoprotein particles (RNP) from pea seedlings are capable of producing a net increase in protein (as measured by Lowry color (J. Biol. Chem. 193:265, 1951)) when incubated aerobically with phosphate, Mg and ATP. It is found now that these particles, unlike those from a number of other sources, contain a high concentration of ninhydrin-positive material (2.5 μmoles alanine equivalents per mg. protein), which decreases in amount when the particles are incubated, and hence, presumably, serves as a substrate for protein synthesis. When the size of the substrate pool is reduced by dialysis, by washing, or by preincubating the particles, the net increase in protein is considerably enhanced by the addition of a mixture of amino acids. Washing is the most effective of these procedures. There is practically no change in protein when washed RNP are incubated in the absence of an added mixture of amino acids. In addition to amino acids, ATP and Mg, the washed RNP also require GTP for activity. The relationships between the incorporation of a labeled amino acid into protein and the net increase in protein under various conditions will be discussed.

4 *Biochemistry*

STUDIES OF SOME SUBCELLULAR COMPONENTS OF BOVINE PANCREAS. Elaine Cohen* and Patricia J. Keller. Dept. of Biochemistry, University of Wash. School of Medicine, Seattle, Wash.

Bovine pancreatic glands were homogenized in 30% sucrose and fractionated by differential centrifugation into several subcellular components. A fraction containing the zymogen granules was analyzed chromatographically using the anion-adsorbent, DEAE-cellulose, and the cation exchanger, IRC-50. The protein composition of the zymogen granules was compared with the composition previously reported for bovine pancreatic juice and found to be qualitatively and quantitatively similar. The microsomal fraction and its subfractions produced by treatment with sodium deoxycholate were studied. The detachment of ribonucleoprotein particles from the microsomes effected by deoxycholate was followed in the analytical cell of the Spinco Model E ultracentrifuge. The ribonucleoprotein particles so obtained were studied with respect to their sedimentation behavior in various ionic media. The distribution at the microsomal level of several pancreatic zymogens was also studied.

5 *Biochemistry*

PROTEIN SYNTHESIS FROM AMINO ACIDS BOUND TO PURIFIED S-RNA. George C. Webster, Dept. of Ag. Biochemistry, Ohio State Univ., Columbus, Ohio.

Amino acid-acceptor RNA (sRNA), prepared by the phenol procedure, has been fractionated with ethanol. Fractions obtained at ethanol concentrations below 50 per cent have little or no ability to bind amino acids. Higher ethanol concentrations precipitate sRNA fractions which bind amino acids, and one fraction ("purified sRNA") binds more than 12 times the amount of amino acid (per mg. sRNA) bound by unfractionated sRNA. When a mixture of amino acids bound to the purified sRNA is incubated with isolated ribonucleoprotein particles, a net synthesis of non-particulate protein occurs. The total amount of protein formed is small, but the amino acids bound to purified sRNA are incorporated into newly-formed "soluble" protein considerably more efficiently than is an equivalent amount of free amino acids. Protein synthesis from an amino acid-sRNA mixture requires rather sharply-defined concentrations of GTP and either magnesium or manganese ions. Preliminary experiments indicate a metabolic role for GTP in the protein synthesis process.

6 *Biochemistry*

INCORPORATION OF AMINO ACIDS INTO GASTRIC PROTEINS OF THE RABBIT. M. A. Karasek (intro. by Louis Shuster). Tufts Univ. Sch. of Medicine, Boston, Mass.

A method for the isolation of rabbit pepsin from rabbit gastric mucosa has been developed. Chromatography on DEAE-cellulose and analysis by moving boundary electrophoresis indicate the presence of more than one component in rabbit gastric tissue with proteolytic activity at pH 1.8. Studies of the incorporation of several radioactive amino acids into the particulate fractions of rabbit stomach indicate that mitochondria, microsomes, and nuclei incorporate amino acids into their respective proteins. Sonication of a homogenate of gastric tissue in a modified Krebs-Ringer medium did not destroy the ability of the homogenate to incorporate amino acids into mucosal proteins. A partial purification of the amino acid incorporating system has been achieved by ammonium sulfate fractionation. ATP and an energy generating system stimulate incorporation into gastric proteins. Ninety-five percent of the incorporated amino acids are internal as determined by reactivity with fluorodinitrobenzene.

7 *Biochemistry*

AMINO ACID UPTAKE BY LIVER PROTEIN OF INTACT AND ADRENALECTOMIZED RATS DURING TRYPTOPHAN PYRROLASE INDUCTION. Olga Greengard (intr. by E. Hirschberg) Dept. of Biochem., College of Phys. & Surgs., Columbia Univ., New York

It has been confirmed that rats, showing increased liver tryptophan pyrrolase levels three hours following tryptophan administration, have more radioactivity in their total liver proteins after injection of C^{14}-amino acids. In order to test whether this reflects increased protein synthesis by one or more subcellular fractions, the radioactivity of liver microsomal, mitochondrial, nuclear and soluble proteins were determined as a function of time after the injection of C^{14}-amino acid. Following 10-60 minutes of incorporation, the specific activities of all four liver protein fractions of the tryptophan adapted animal were above the corresponding control fractions. The soluble fractions showed the most striking difference. Experiments with tryptophan induction in adrenalectomized rats showed stimulated C^{14}-leucine uptake into all cell fractions after 15-20 minutes of isotope incorporation while after 60-80 minutes of incorporation this stimulatory effect has disappeared. Since the enzyme tryptophan pyrrolase represents a very small fraction of the liver proteins, it is likely that these observations are at least partly due to general effects of tryptophan on protein synthesis.

8 *Biochemistry*

THE THERMAL LABILITY OF RAT LIVER MICROSOMES AND RIBONUCLEOPROTEIN-PARTICLES. K. Hanson+ and R. Rendi (intr. by C. Gilvarg), Dept. of Biochemistry, New York Univ. Col. of Medicine, New York, N. Y.

Rat liver microsomes when pre-incubated in vitro at 37° lose their ability to incorporate C^{14}-leucine into proteins when subsequently incubated for 20 minutes with cell sap, an ATP generating system and the radioactive amino acid.

This decay process was followed to the point where about 80% of the original activity was lost. The log of the incorporated radioactivity, i, when plotted against the time of preincubation, t, gave a curve showing a marked break and corresponding to the form $\log i = \log (A e^{-k_1 t} + B e^{-k_2 t})$. Similar results were obtained with ribonucleoprotein-particles isolated from the same tissue. These findings suggest that two species of particles are present in the preparations, or that two mechanisms with different rate limiting steps are involved.

The rate of decay of microsomes was independent of concentration over a 4-fold dilution. Ribonucleoprotein-particles were observed to be stabilized at higher concentrations. The stability of ribonucleoprotein-particles is little affected by K^+ and Mg^{++}, but is decreased by mercaptoethylamine.

+ Jane Coffin Childs post-doctoral fellow.

XUM

9 *Biochemistry*

AMINO ACID INCORPORATION INTO PROTEIN BY EXTRACTS OF E. COLI. M. Lamborg (intr. by R. B. Loftfield.) Huntington Labs. of Harvard Univ. at Mass. Gen. Hospital, Boston, Mass.

Extracts of E. coli prepared by grinding whole cells with alumina are capable of incorporating amino acids into hot TCA precipitable protein. The incorporation into crude extracts is dependent upon ATP (1 mM) and an ATP generating system, GTP (0.25 mM) and Mg^{++} (7×10^{-3} M). It is stimulated by the addition of KCl (6×10^{-2} M) and an amino acid mixture (0.2 mM each of 18 amino acids) and inhibited by ribonuclease (0.01 γ/ml) and chloramphenicol (30 γ/ml). The site of initial incorporation is the bacterial ribonucleoprotein particle. Addition of the 105,000 x g supernatant solution stimulates the incorporation; large bacterial fragments are not required for incorporation. It is essential that whole cell contamination be less than 1×10^5 whole cells/ml at the start of a 15 min incubation. The incorporating system of the crude bacterial extract is very similar to the rat liver system originally described by Zamecnik and Keller.

10 *Biochemistry*

ENRICHMENT OF SPECIFIC ACTIVITY OF AMINOACYL RNA. P. C. Zamecnik and M. L. Stephenson.* Huntington Labs. of Harvard U. at Mass. Gen. Hosp., Boston, Mass.

RNA prepared by phenol extraction of yeast was treated with alkali at pH 10 to remove esterified amino acids, then was labeled specifically with C^{14}-L-valine by means of an activating enzyme fraction. The unesterified terminal 2'3' hydroxyl groups of the RNA molecules not bearing amino acid were oxidized to dialdehydes by periodate. An aldehyde addition reaction with naphthoic acid hydrazide was carried out, similar to that of Ashbel, R., and Seligman, A., (Endocrinology 44, 565, 1949). Tetrazotized o-dianisidine was coupled to the resulting RNA carbonyl-hydrazide compound by reaction in 50% ethanol, pH 4, 0°, 10'. The RNA thus acquired a rich, bluish purple color. This RNA, in 2.2 M phosphate buffer pH 7.5, was precipitated at a two-phase interface by gradual addition of n-propanol, a series of fractions being thus obtained. The dye-bound RNA precipitated from solution at a lower propanol concentration than did the non-dye bound, amino acid-labeled RNA, with a resulting elevation of specific activity of the C^{14}-valine-RNA. The solubility of dye-bound RNA is also altered in several solvent systems amenable to countercurrent distribution fractionation. This type of aldehyde addition procedure has potential general applicability to the problem of separating individual species of S-RNA molecules.

11 *Biochemistry*

PEPTIDE SYNTHESIS WITH MICROSOMAL AMINOPEPTIDASE OF SWINE KIDNEY. C. H. O'Neal (intr. by Francis Binkley) Dept. of Biochemistry, Emory University, Atlanta, Ga.

During studies of the reaction of a dilute acid hydrolysate of glutathione, containing cysteinylglycine, with swine kidney peptidase, prepared by the procedure of Binkley et. al., C^{14}-glycine was added to the incubation media. After paper electrophoresis of the reaction products at 1000v in pH 8 phosphate buffer considerable radioactivity (up to 30%) could be found in the area of the cysteinyl glycine band adjacent to that of glycine. Incubation of the enzyme alone with C^{14}-glycine resulted in significant labeling of the enzyme. In further experiments the system was simplified by using cysteine, labeled glycine, enzyme, and buffer. N-ethylmaleimide caused a pronounced change in the electrophoretic mobility of the cysteinylglycine giving an excellent separation from glycine. When N-ethylmaleimide was incubated with the reaction mixture the cysteinylglycine band contained significantly larger amounts of radioactivity; this effect was probably due to the effect of product removal on the equilibrium.

12 *Biochemistry*

THE ROLE OF PEPTIDES IN THE SYNTHESIS OF STREPTOCOCCAL PROTEIN. Eugene N. Fox* and L. O. Krampitz. Dept. of Microbiology, Western Reserve Univ. School of Medicine, Cleveland, Ohio.

It has been previously shown that peptides from various sources are growth factors for streptococci. Work in this laboratory has demonstrated that the de novo synthesis of the M protein of group A hemolytic streptococci is strongly stimulated by the presence of peptides in nonproliferating trypsinized cells. Data is presented now which demonstrates that peptides from enzymatically digested casein enhance the incorporation of free C^{14}-labelled amino acids into the M protein antigen and cellular proteins. These peptides also stimulate the synthesis of streptolysin O in resting cells. Protein synthesis in the presence of peptides is dependent on free amino acids and a source of energy; it is inhibited by chloramphenicol. Peptides, prepared from enzymatically digested proteins of Rhodospirillum rubrum grown photosynthetically in C^{14} bicarbonate, also stimulate the synthesis of streptococcal protein and are incorporated to a small but significant extent into the M protein antigen. Glutamine and asparagine will partially substitute for the peptides. Peptides prepared by incomplete acid hydrolysis of casein are inactive. Work is in progress to determine if intact peptide bonds are retained during the incorporation into the M protein.

13 *Biochemistry*

STAGES IN HEMOGLOBIN SYNTHESIS IN A CELL-FREE SYSTEM. J. Bishop,* E. Allen,* J. Leahy,* A. Morris* and R. Schweet. Dept. Biochem., City of Hope Med. Center, Duarte, Calif.

Previous studies have shown that incorporation of C^{14}-amino acids into protein, using ribosomes from rabbit reticulocytes plus soluble enzymes and cofactors, represents mainly hemoglobin synthesis. Stages in hemoglobin synthesis were studied starting with free C^{14}-amino acids, with isolated C^{14}-amino acid-RNA compounds, or with ribosomes containing incorporated C^{14}-amino acid. Incorporation using the complete system is increased 2-3 fold by added transfer RNA. Incorporation starting with C^{14}-amino acid-RNA requires a partially purified enzyme fraction and guanosine triphosphate. The enzyme used does not catalyse incorporation starting with free C^{14}-amino acid. Amino acid-RNA from either guinea pig liver or rabbit reticulocytes can be used. Maximal incorporation can be obtained without added ATP, and under these conditions no incorporation into soluble protein is observed. When ribosomes labeled in this way, or in the complete system, are incubated with enzyme and ATP, soluble labeled hemoglobin is formed. The enzymatic reaction requires Mg ion and is inhibited by PCMB, in contrast to non-enzymatic release of soluble protein by chelating agents. Reticulocyte ribosomes, therefore, contain the precursors for and are the site of hemoglobin synthesis.

14 *Biochemistry*

FURTHER STUDIES WITH AMINO ACID LIPID COMPLEXES FORMED IN HEN OVIDUCT DURING PROTEIN SYNTHESIS. R.W. Hendler, Natl. Heart Inst., Bethesda, Md.

Upon short term incubation of amino acid with intact cells of hen oviduct, there are formed lipid-soluble amino acid complexes. Countercurrent distribution and chromatography of this material on columns of alumina-silica, and on silicic acid has yielded reproducible patterns. The more nonpolar compounds are essentially not produced when radioactive amino acid is added to an alcohol ether extract of the tissue prior to the usual isolation. The most nonpolar material is present in higher percentage in the lipids from the particulate than from the supernatant fractions of the tissue homogenate. 2,4-dinitrophenol increases the total amount of lipid-soluble radioactivity, but the chromatographic distribution is markedly altered. That is, a lesser per cent of radioactivity is found in the most nonpolar material of the particulate fraction and a greater per cent is present in a more polar material from the supernatant. The formation of the complexes has been observed with each of eleven amino acids including acidic, neutral and basic types. Similar types of metabolically active amino acid-lipid complexes have been found in the ribosomes and cell wall of E. coli. The latter have been fractionated to yield chromatographically distinct entities.

15 *Biochemistry*

PURIFICATION OF A SUPERNATANT FACTOR REQUIRED FOR TRANSFER OF sRNA-AMINO ACID TO LIVER MICROSOMES. Daniel Nathans and W. C. Hülsmann (intr. by Fritz Lipmann). The Rockefeller Institute, New York, N.Y.

A resolution of the system responsible for amino acid transfer from sRNA to rat liver microsomes was attempted. In our hands, microsomes washed with sucrose solution showed only a sulfhydryl requirement, supernatant having little additional effect. On washing with 0.5% desoxycholate (DOC), particles were obtained that required for activity a heat-labile, non-dialyzable factor present in the 105,000xg supernatant and in the DOC wash. DOC-particles were used to assay this factor. A 30-fold concentration with 30% recovery was obtained by ammonium sulfate-acetone fractionation. In addition to this fraction, added glutathione, GTP, and a nucleoside-triphosphate-generating system are needed for full activity. In a representative experiment, from 1300 c.p.m. C^{14}-leucyl-sRNA (E. coli), 370 c.p.m. were transferred in 5 minutes to liver DOC particles in the presence of 30 µg of the purest supernatant fraction, against a value of 22 c.p.m. in its absence. In the liver particle system, E. coli-sRNA was as good as or better than liver sRNA. The microsome preparation used here has proven to be rather stable to lyophilization. (Supported by a grant from the National Cancer Institute, National Institutes of Health, U. S. Public Health Service.)

16 *Biochemistry*

A RELATIONSHIP BETWEEN THE INTRACELLULAR CONCENTRATION OF LEUCINE-C^{14} AND ITS INCORPORATION INTO GLOBIN IN THE DUCK ERYTHROCYTE. Donald S. Wiggans, William W. Burr, Jr., and Herbert W. Rumsfeld, Jr.* Department of Biochemistry, Univ. of Texas, Southwestern Med. School, Dallas.

Duck erythrocytes have been incubated with DL-leucine-1-C^{14} in a medium suitable for the incorporation of amino acids into globin. After 80 minutes, the cells were washed and resuspended for a second incubation in a medium which contained no labeled leucine. The radioactivity of the cell fell rapidly within 1 to 2 hours to a level which remained essentially constant thereafter although the specific activity of the globin continued to rise for at least 20 hours to a level up to three times that which existed at the initiation of the second incubation. The increase in specific activity was not inhibited either by anoxia or the lack of glucose. The addition of an amino acid mixture devoid of leucine did not enhance the process. The increase in specific activity for many hours in the absence of any additional external source of labeled leucine is a striking property of the cell which suggests the nature of the process of incorporation of amino acids into protein in the erythrocyte. (Supported by grants from the U. S. Atomic Energy Commission and the Robert A. Welch Foundation, Houston, Texas.)

17 *Biochemistry*

ENZYMATIC SYNTHESIS OF THE PEPTIDE IN A URIDINE NUCLEOTIDE IN S. AUREUS. Jack L. Strominger and Eiji Ito*. Dept. of Pharmacology, Washington U. Sch. Med., St. Louis, Mo.

A uridine nucleotide which accumulates in S. aureus inhibited by several antibiotics is represented as UDP-GNAc-lactyl-(L)ala-(D)glu-(L)lys-(D)ala-(D)ala. Since accumulation of nucleotides containing incomplete peptides can be induced under other circumstances, it has been inferred that the peptide is synthesized by stepwise addition of amino acids. Each of the enzymes catalyzing the stepwise addition to UDP-GNAc-lactic of 1. (L)ala, 2. (D)glu, 3. (L)lys and 4. (D)ala-(D)ala, as well as the enzyme catalyzing the synthesis of the dipeptide, (D)ala-(D)ala, has now been separated and partially purified. Each of these reactions requires ATP and a divalent cation. ADP and Pi are the products formed from ATP by the lysine adding enzyme, and its mechanism has been investigated. The mechanisms of the other reactions are under study. The antibiotic, oxamycin, owes its selective toxicity for microorganisms to competitive inhibition both of alanine racemase and of the dipeptide synthesizing enzyme (J. Amer. Chem. Soc., in press). (Supported by NSF Grant 7619 and NIAID Grant E-1902).

18 *Biochemistry*

H_2O_2-INDUCED CATALASE SYNTHESIS IN RHODOPSEUDOMONAS SPHEROIDES. Roderick K. Clayton (intr. by S. F. Carson) Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee.

H_2O_2 induces catalase synthesis in R. spheroides. Maximal induction is caused by sustained H_2O_2 concentration, 1-2 µM; 0.05 µM has no measurable effect. In a mature culture, H_2O_2 added initially to a concentration of 40 µM is rapidly destroyed; after 2 min, $[H_2O_2] < 0.05$ µM. Catalase synthesis in response to this addition of H_2O_2 begins after a 7 min lag and continues for 30 min. The response therefore occurs long after external inducer has been dissipated. An induced state, or internal inducer (I_1), has been generated and persists about 30 min (many hours at 0°C.). With an H_2O_2-treated culture, chilled to 0° after 5 min, restored to room temperature after 5 hr, a nearly maximal response occurs. Formation of I_1 requires energy and does not involve a reaction between H_2O_2 and catalase. Sodium azide (3 µM) inhibits catalase and thus prolongs the lifetime of the H_2O_2 added initially. More induced catalase synthesis results, implying that more I_1 was formed. Azide added 5 min after the H_2O_2 (i.e., after the H_2O_2 is dissipated and the formation of I_1 is complete) also increases the response. Azide appears, therefore, to retard the dissipation of I_1. This result suggests that I_1 is bound H_2O_2, susceptible to slow attack by catalase, or in equilibrium with free H_2O_2.

19 *Biochemistry*

INDUCIBILITY OF PENICILLINASE IN STAPHYLOCOCCUS AUREUS. Harry G. Steinman, Natl. Insts. of Health, Bethesda, Md.

Evidence for the inducibility of staphylococcal penicillinase was obtained in previous work which revealed a rapid increase in the rate of enzyme formation in cultures exposed to high concentrations of penicillin, as determined by continuous measurement of penicilloic acid formation in the Warburg apparatus. Studies on cells inactivated with PCMB have now shown that the rate of enzyme formation was dependent on the concentration of inducer and that the increase in rate was linear. More direct evidence for the induced formation of penicillinase was obtained from studies on cell-free preparations produced with the Nossal disintegrator. Over 50% of the enzyme activity of whole organisms was recovered in the cell-free extracts. After exposure of the bacteria to penicillin, there was observed a 10-fold increase in the penicillinase activity of the soluble protein fraction. The relative total activities of extracts from uninduced and induced cells was maintained through various purification procedures. Approximately 1000-fold concentration of specific enzyme activity of the protein fraction was achieved, but, by comparison with purified penicillinase derived from Bacillus cereus, it was still relatively impure. The present data support the view that penicillinase is an inducible enzyme of S. aureus.

20 *Biochemistry*

KINETICS OF PROTEIN SYNTHESIS IN GROWING YEAST. H. K. Kihara, R. J. Young and H. O. Halvorson*. Dept. of Bacteriology, University of Wisconsin, Madison

Various lines of evidence have indicated the presence of transient complex intermediates in protein synthesis. In order to further demonstrate these in yeast, the initial kinetics of incorporation of $S^{35}O_4^=$ into yeast proteins was examined. Following the addition of $S^{35}O_4^=$ to an exponentially growing culture, a rapid incorporation of label into the hot TCA insoluble fraction occurred during the first 10 seconds. This was followed (10 to 20 seconds) by a slower steady-state incorporation into total proteins. The initial rate of incorporation is suppressed by prior N starvation or by the addition of unlabled $SO_4^=$. During this period the specific activity of the ribosomal proteins is higher than that of the soluble proteins. These findings are consistent with the view that a transient protein intermediate, which is probably associated with the ribosomal fraction, is synthesized within a few seconds.

XUM

21 *Biochemistry*

INCORPORATION OF AMINO ACIDS INTO PROTEIN OF PARTICULATE PREPARATIONS FROM AZOTOBACTER VINELANDII. D. P. Burma and Maharani Chakravorty, (intr. by Paul Kaesberg). Bose Institute, Calcutta-9, India.

Incorporation of glutamate, glycine and histidine into protein of the particulate preparations from A. vinelandii is dependent on the oxidative phosphorylation carried out by the particles. Added ATP is inhibitory to glycine incorporation. The amino acids were mostly incorporated within the protein chain but were partly associated with the nucleic acid of the particles. The incorporation was completely suppressed by hydroxylamine. The effect of a number of antibiotics and antimetabolites on both in vivo and in vitro amino acid incorporation has been studied and the possibility of cell-wall synthesis in particulate preparations has been ruled out. Amino acid mixtures are inhibitory to the incorporation system. The particulate preparations seem to have all the components necessary for protein synthesis which remain unaffected by repeated washings with buffer or by dialysis. Extraction with NaCl, however, leads to the loss of incorporating ability which can be restored by the addition of pH 5 enzyme from rat liver. The supernatant but not the particulate fractions of A. vinelandii can catalyze the activation of almost all the amino acids tested.

22 *Pharmacology*

SUPPRESSION OF UPTAKE OF L-ARGININE-U-C^{14} INTO NUCLEAR PROTEINS BY 5-BIS(2-CHLOROETHYL) AMINOURACIL. Joseph R. Davis,* George R. Honig* and Harris Busch. Dept. of Pharmacol., Univ. of Ill., Coll. of Med., Chicago, Illinois.

5-bis(2-chloroethyl) aminouracil markedly inhibited the uptake of L-arginine-U-C^{14} into nuclear proteins of the Walker tumor and spleen (Busch, Amer and Nyhan, J. Pharm. 127, 195, 1959). To determine specific inhibition of biosynthesis of particular acid-soluble nuclear proteins obtained by chromatography on CM-cellulose (Davis and Busch, Cancer Res. 19, 1157, 1959) L-arginine-U-C^{14} was injected I.P. into tumor-bearing rats at varying intervals following administration of 4 mg/kg of the mustard; 1 hr later the tissues were excised. The acid-soluble nuclear proteins were chromatographically resolved into 2, 3 and 5 radioactive fractions in tumor, liver and spleen. The mustard suppressed C^{14} uptake to 45% and 18% of control values in the first radioactive peak of the tumor at 6 and 24 hrs. respectively, after administration of the drug, and at corresponding times suppressed C^{14} uptake to 62% and 36% of control values in the second peak. C^{14} uptake was suppressed to less than 20% of control values in earlier peaks of spleen by 6 hrs. after treatment. These data indicate that aminouracil mustard exerts a broad spectrum of inhibitory effect against incorporation of L-arginine-U-C^{14} into acid-soluble nuclear proteins of tumor and spleen with some selectivity of inhibition of biosynthesis of particular nuclear proteins.

23 *Pharmacology*

INHIBITION OF UPTAKE OF L-ARGININE-U-C^{14} INTO ANIONIC NUCLEAR PROTEINS OF THE WALKER TUMOR BY A NUMBER OF CARCINOSTATIC AGENTS. Allen Lipsey*, Samir M. Amer* and Harris Busch. Dept. of Pharmacol, University of Illinois, Coll. of Med., Chicago, Illinois.

It has previously been shown that 5-bis(2-chloroethyl) aminouracil inhibits uptake of arginine-U-C^{14} into the anionic nuclear proteins of the Walker 256 carcinosarcoma (Busch, Amer and Nyhan, J. Pharm. 127, 195, 1959). Rats bearing the Walker 256 carcinosarcoma were treated with HN2 (2 mg/kg), Chlorambucil (15 mg/kg), Myleran (15 mg/kg), 5-fluorouracil (25 mg/kg) and 6-mercaptopurine (50 mg/kg). Twenty-three hrs. after treatment, the rats were injected with 10 uc of L-arginine-U-C^{14}. One hr. later various cellular fractions were isolated. The specific activity of the anionic nuclear proteins of the tumor was reduced to 79,62,34,54,49% of the control values respectively. In the spleen, the specific activity of these proteins was reduced to 38,26,43,29 and 50% of the controls respectively. In the liver, the corresponding values were 84,87,47,52,61% of the controls. The incorporation of the labeled amino acid into the other cellular fractions of three tissues was not affected by these drugs after 48 hrs. of treatment. From these studies, it would appear that a common metabolic effect of a number of antitumor agents is inhibition of the uptake of L-arginine-U-C^{14} into anionic nuclear proteins.

219. PROTEINS, POLYPEPTIDES—Synthesis—Amino Acid Activation

1 *Biochemistry*

INCORPORATION OF PHENYLCYSTEINES INTO PROTEINS. John T. Smith* and John L. Wood. Div. of Chemistry, Univ. of Tennessee Medical Units, Memphis, Tenn.

Intermediate steps in the formation of mercapturic acids by conjugation of halobenzenes with tissue sulfur compounds have been studied in vivo and in vitro. Isolation of radioactive proteins and peptides from the livers and intestines of rats fed iodobenzene-I^{131} has been reported previously, Fed. Proc., 17, 313 (1958). Further fractionation of the tissue proteins has shown a correlation between their specific activity and their RNA content. In addition, separation of cellular matter into particulates and pH 5 enzyme has shown the highest specific activity to be associated with the pH 5 enzyme fraction. When chlorophenylcysteine S^{35} was incubated with the pH 5 enzyme (Keller and Zamecnik, J. Biol. Chem., 221, 45-59 (1956)), significant incorporation into both the RNA and protein of the pH 5 enzyme system was observed. The activation of chlorophenylcysteine by the pH 5 enzyme system was confirmed by demonstrating that a hydroxamate was formed when chlorophenylcysteine was incubated with the enzyme according to the method of Hoagland, et al, J. Biol. Chem., 218, 345 (1956). However, this system was not capable of conjugating chlorobenzene-C^{14} with protein. Some implications of these findings to the theory of mercapturic acid formation will be discussed.

2 *Biochemistry*

ALTERATION IN AMINO ACID ACTIVATION WITH FUNCTIONAL DEVELOPMENT OF THE RAT MAMMARY GLAND. Edsel T. Bucovaz and J. Wendell Davis (intr. by Edward A. Doisy.) St. Louis Univ. Sch. of Med., St. Louis, Mo.

The amino acid carboxyl-activation reaction, as reflected in a stimulation of the PP^{32}-ATP exchange rate, has been studied in mammary tissue of virgin, pregnant, and lactating female rats. The PP-ATP exchange in the supernatant fraction, 104,000 x g., of the virgin mammary gland was not measurably stimulated on addition of any of a number of amino acids tested. This same fraction from pregnant and lactating animals showed definite amino acid stimulated exchange activity. Greater rates of amino acid activation could be measured in preparations of mammary gland from lactating rats than from pregnant (but non-lactating) rats. It appears that in the hormone induced proliferation of mammary tissue, followed by the hormone induced lactation, there is a progressive increase in the rate of activation of many of the amino acids.

3 *Biochemistry*

COUNTERCURRENT DISTRIBUTION OF AMINO ACID-ACCEPTOR RIBONUCLEIC ACIDS. Robert W. Holley and B. P. Doctor*. U.S. Nutrition Lab. Agricultural Research Service, and Dept. of Biochem. and Nutr., Cornell Univ., Ithaca, N.Y.

Countercurrent distribution, using the solvent system of Warner and Vainberg (Fed. Proc. 17:331 (1958)), gives fractionation of the specific amino acid-acceptor RNAs obtained from the "soluble" fraction of rat liver homogenate (J. Am. Chem. Soc. 81:753 (1959); Biochim. et Biophys. Acta 35:272 (1959)). In these experiments the recovery of activity was poor (40%), the losses being due in part to the high pH of the solvent system. A number of modifications have now been made in the procedure, including the use of a solvent system adjusted to pH 6 and containing 0.001 M $MgCl_2$, the substitution of a more rapid dialysis procedure in the presence of dilute $MgCl_2$, and the use of a rotary evaporator in place of lyophilization. Using this new procedure, amino acid-acceptor RNAs prepared from the "soluble" fraction of rat liver homogenate and from yeast were subjected to countercurrent distribution. After 200 transfers, the threonine-, histidine-, leucine-, and tyrosine-acceptor RNAs gave peaks with successively higher partition coefficients. The recovery of activity was approximately 80%. The tyrosine-acceptor RNA was reisolated with a specific activity six times that of the original preparation. (Supported in part by a grant from the National Science Foundation.)

4 *Biochemistry*

EFFECT OF NITROUS ACID ON AMINO ACID ACCEPTOR RIBONUCLEIC ACIDS. Mario A. Marini (Intr. by H. H. Williams.) Dept. of Biochem. Cornell Univ. and U. S. Nutrition Lab. Ithaca, N.Y.

Nitrous acid has been shown to exert an effect on tobacco mosaic virus (Z. Naturforsch. 13b;697, 1958) and on bacteriophages φX174 and T4 (Virology, 9:375, 1959). The mutations presumably result from a selective deamination of the base components of the nucleic acid leading to alterations in the base sequence. According to present concepts of protein synthesis, there is a different amino acid acceptor ribonucleic acid involved in the transfer of each of the twenty different amino acids from the activating enzyme to the template ribonucleic acid. The specificities of the amino acid acceptor ribonucleic acids presumably involve, in some way, a specific and characteristic sequence of bases. It might be anticipated, therefore, that nitrous acid would variably affect the different amino acid acceptor ribonucleic acids, depending on the nature of the bases that confer specificity and on their relative reactivity. Amino acid acceptor ribonucleic acids, obtained from yeast, were reacted with 0.133M nitrous acid at pH 3.7 and 25°C. At various times the reaction was stopped by neutralizing the acid. After dialysis, the treated nucleic acids were assayed for activity. After one hour, with nitrous acid, the following activities remained in the nucleic acids; tyrosine, 0.9, threonine and histidine, 0.5 and leucine, 0.3.

5 *Biochemistry*

EVIDENCE FOR INTERMEDIATE IN ENZYMATIC SYNTHESIS OF GLUTAMINE. P.R. Krishnaswamy*, Vaira Pamiljans*, and Alton Meister. Dept. Biochem. Tufts Univ. Sch. Med., Boston.

Highly purified sheep brain glutamine synthesis enzyme was protected from heat denaturation by ATP + Mg^{++} (not by ATP alone, ADP + P_i, GTP, glutamine, etc) suggesting specific combination of ATP with enzyme in absence of glutamate and NH_3. Incubation (10 min, 37°) of NH_3-free enzyme (10 mg), C^{14}-L-glutamate, Mg^{++}, ATP, followed by heating (100°, pH 7.2, 5 min) gave pyrrolidone carboxylate (PC; identified chromatographically in 4 solvents and by conversion to glutamate with HCl) in amount of same order as enzyme; no glutamine was detected. Much less PC was formed when ATP, Mg^{++}, or enzyme were separately omitted, with heat-denatured enzyme, or if heating at 100° was omitted. Incubation as above plus NH_3 followed by heating as above also gave much less PC, but gave glutamine. (Glutamine is not significantly converted to PC at pH 7.2, 100°, 5 min). Results are consistent with formation of a γ-carboxyl-activated glutamate intermediate complex involving enzyme, Mg^{++}, and ATP which may (a) be converted to PC by heating, or (b) react with NH_3 to give glutamine.

6 *Biochemistry*

AMINO ACID ACTIVATION AND CARRIER RNA IN CELL NUCLEI. I.W. Hopkins*, V.G. Allfrey, and A.E. Mirsky. Rockefeller Institute, New York.

An enzyme fraction precipitable at pH 5 catalyzing the amino acid dependent exchange of P^{32} pyrophosphate with ATP is found in calf thymus nuclei prepared in nonaqueous media or in isotonic sucrose. 15 L-amino acids stimulate this exchange. Similar amino acid activating enzymes can be extracted from nonaqueous chicken kidney nuclei and calf thymus and liver nuclei prepared in 2.3 M sucrose. The calf thymus nuclear pH 5 fraction incorporates amino acid into some nuclear RNA's in the presence of Mg and ATP. RNAase treatment of leucine-C^{14} labeled nuclear RNA gives an acid soluble fragment separable from the free amino acid by chromatography and from which the amino acid is released by base. Nuclear RNA from calf liver accepts amino acids from thymus nuclear activating enzymes and thymus nuclear RNA accepts from enzymes prepared from whole liver. Chloramphenicol (6.7×10^{-3} M), which blocks protein synthesis in thymus nuclei (Breitman and Webster, Biochem. et Biophys. Acta, 27, 409 1958), has no effect on the nuclear synthesis of ATP, amino acid activation, or transfer to RNA.

7 *Biochemistry*

SYNTHESES OF 2'(3')-DL-PHENYLALANYL ESTERS OF RIBONUCLEOSIDES. David H. Rammler* and H.G. Khorana, British Columbia Research Council, University of B. C., Vancouver, Canada.

Recent work on the small molecular weight ribonucleic acids carrying α-amino acyl groups on the terminal residues has focussed interest on the amino acyl esters of ribonucleosides and, consequently, a study of the synthesis and properties of such esters has been undertaken. When 5'-O-tri-(p-methoxyphenyl)-methyluridine (M. Smith and H.G. Khorana, unpublished work) is reacted with crystalline DL-carbobenzoxyphenylalanine anhydride, the major product is 2'(3')-DL-carbobenzoxyphenylalanyl-5'-O-tri-(p-methoxyphenyl)-methyluridine. Removal of the tri-(p-methoxyphenyl) methyl group by mild acid followed by chromatography affords the pure 2'(3')-carbobenzoxyphenylalanyluridine. Hydrogenolysis of the latter yields pure phenylalanine esters. The condensation of 5'-O-triphenyl methyladenosine with DL-carbobenzoxyphenylalanine anhydride in pyridine gives several nucleoside containing products, separable by chromatography. One of the major products has been tentatively identified as the 5'-O-triphenylmethyl, 2'(3')-DL-carbobenzoxyphenylalanyladenosine. Removal of the triphenylmethyl group by hydrogen bromide followed by hydrogenolysis affords electrophoretically homogeneous phenylalanine esters. Some properties of the amino acyl esters will be reported.

8 *Pathology*

MORPHOLOGIC HISTOCHEMICAL STUDIES OF AMINOPEPTIDASE IN AUTOPSY SPECIMENS. Mitchell Rosenholtz* and Lee Wattenberg, University of Minnesota Medical School, Minneapolis.

Aminopeptidase activity has been studied in human and animal tissues at various intervals after death. The enzyme activity was found to persist for many hours postmortem without demonstrable change as estimated from microscopic observations. This stability make it possible to study aminopeptidase activity in a variety of normal and pathologic tissues at autopsy. A number of malignant neoplasms have been found to have aminopeptidase activity. Thus 15 of 17 specimens of gallbladder and biliary duct carcinomas showed activity. Gastric and breast carcinomas have been less consistently active while only one of ten specimens of carcinoma of the pancreas was positive. Colon carcinomas have been uniformly negative. Studies of non-neoplastic tissues have concentrated on the liver. Normal bile ducts and proliferating bile ducts of cirrhotic livers showed very intense activity, whereas hepatic cord cells showed only minimal to moderate activity. These differences have been used to distinguish proliferating bile ducts from small cords of hepatic parenchymal cells seen in the scars of cirrhotic livers.

220. PROTEINS, POLYPEPTIDES—Synthesis—DNA and RNA

1 *Biochemistry*

TRYPTOPHAN PYRROLASE INDUCTION AND RNA METABOLISM. P. Feigelson, Dept. of Biochemistry, College of Physicians and Surgeons, Columbia University, New York City.

Tryptophan administration results in an elevation of rat liver tryptophan pyrrolase levels, which is followed several hours later by stimulated incorporation of inorganic P^{32} into RNA. The amount of microsomal RNA per gram liver increases concurrently with this period of accelerated P^{32} incorporation into RNA. Thus, an increased net synthesis of microsomal RNA takes place following tryptophan induction, indicating either the formation of new microsomes or a shift in the RNA:protein ratio of the microsomes. P^{32} incorporation into liver RNA during tryptophan pyrrolase induction by cortisone was studied to determine whether hormonally induced enzyme elevations similarly influence RNA metabolism. 0.5 mg. Cortisone/100 gm. body weight causes increased tryptophan pyrrolase levels reaching fourfold control values after 5 hours with concurrent 180% increases in incorporation of P^{32} into RNA. Following cortisone administration, the RNA turnovers parallel the changes of tryptophan pyrrolase levels, whereas with tryptophan induction the stimulated RNA synthesis occurred several hours after the liver tryptophan pyrrolase levels had returned to control values. While both substrate and hormonal induction stimulate RNA synthesis, their kinetic relationships are markedly different and characteristic.

XUM

2 *Biochemistry*

DIRECT ACTION OF THE GENE IN ENZYME SYNTHESIS
Monica Riley*, Arthur B. Pardee, Francois Jacob* and Jacques Monod*. Univ. of Calif., Berkeley, California and Inst. Pasteur, Paris.

During bacterial conjugation, a gene governing synthesis of β-galactosidase can be transferred into a bacterium previously lacking this genetic capacity. When a cross of this sort was performed with appropriate strains of Escherichia coli, β-galactosidase synthesis commenced within a few minutes after injection of the gene, and proceeded at maximum constant rate in each zygote. Experiments with radioactive bacteria were undertaken to test the possibility that the gene acts by synthesizing a stable intermediate (RNA) which in turn catalyzes the synthesis of the enzyme. Donor bacteria were labeled with P^{32} and were mated with non-radioactive recipients. After the zygotes were fully functioning to produce enzyme they were frozen for various lengths of time during which P^{32} decay occurred and damaged the injected gene. With progressive amounts of P^{32} damage, the ability of the zygote to form β-galactosidase was reduced. Therefore, the gene does not act by synthesizing a stable, autonomous intermediate. Continued integrity of the gene is essential for enzyme synthesis.

3 *Biochemistry*

FACTORS DETERMINING THE SPECIFICITY OF HEMOGLOBIN SYNTHESIZED IN A CELL-FREE SYSTEM. H. Lamfrom (intr. by H. Borsook) Calif. Inst. of Technology, Pasadena, Calif.

Cell-free preparations from rabbit or sheep reticulocytes were used to synthesize hemoglobin characteristic of the species. When microsomes of one animal species were incubated with "pH 5 enzyme" and supernatant from the pH 5 fraction of the other, the hemoglobin formed corresponded to that of the microsome species. C^{14}-leucine and C^{14}-valine were used to identify the newly formed hemoglobins which were separated chromatographically. This lends support to the view that the chromatographic characteristic of the final protein, presumably the amino acid sequence, is determined in the microsomal particle. Further experiments indicated a requirement for material in the supernatant of reticulocytes for hemoglobin synthesis. This fraction could not be replaced by guinea pig liver supernatant, which was, however, active in C^{14}-leucine incorporation into unidentified soluble protein. (Supported by a grant from U. S. Public Health Service)

4 *Biochemistry*

THE BINDING OF OXYHEMOGLOBIN BY RIBONUCLEOPROTEIN FROM A RAT TUMOR. Mary L. Petermann. Sloan-Kettering Inst. for Cancer Research, New York 21, N. Y.

Ribonucleoprotein (RNP) from a rat tumor, the Jensen sarcoma, purified by washing in 0.001 M potassium phosphate-0.0005 M $MgCl_2$ buffer, at pH 7 (Federation Proc., 18, 302 (1959)), still carries excess protein which can be removed by washing at pH 8 and ionic strength 0.1, or by treatment with deoxycholate. For a detailed study of this protein binding, RNP was purified by deoxycholate treatment. Its ability to bind oxyhemoglobin (HbO_2) was studied by electrophoretic analysis and by the spectrophotometric determination of the HbO_2 left in the supernatant after sedimentation of RNP-HbO_2 mixtures for 90 minutes at 105,000 x g. At pH 7, in the 0.001 M phosphate- 0.0005 M $MgCl_2$ buffer (ionic strength 0.0035), one mg of RNP could bind up to 0.8 mg of HbO_2. On the addition of small amounts of KCl, the binding decreased markedly; at ionic strengths of 0.02, 0.03 and 0.05 the ratio of bound to free HbO_2 fell to 36, 20 and 3% of the value at ionic strength 0.0035. At pH 8 and ionic strengths of 0.02 or 0.1, no binding was observed by the sedimentation method, but the complexity of the electrophoretic patterns obtained at ionic strength 0.1 indicated some interaction. This ability of RNP to bind protein should be considered in the design of purification procedures. It may also be related to the functioning of RNP as a template in protein synthesis.

5 *Biochemistry*

AMINO ACID INCORPORATION INTO MICROSOMAL PROTEINS OF NORMAL AND MALIGNANT CELLS. Harold T. Shigeura and Charles N Gordon*, Merck Institute for Therap. Res., Rahway, N. J.

The 20,000 x g supernatant fluids of normal and neoplastic cells containing equal concentrations of microsomal proteins were incubated at 37°C with radioactive amino acids and a source of energy. The initial rates of incorporation of L-leucine C^{14} into the RNP particles of liver and hepatoma were very rapid and about equal. However, the rate of incorporation into hepatoma microsomes was about 3-5 times greater than that of liver microsomes and equal to that of the RNP particles. Similar results were obtained with L-valine C^{14} and L-lysine C^{14}. The rates of incorporation into regenerating liver and Murphy lymphosarcoma were greater than those of liver and spleen. Tests with various combinations of microsomes and cell sap showed that the soluble fractions of liver and hepatoma are interchangeable. The cofactor requirements of regenerating liver and hepatoma were found to be similar to that of liver. These results suggest that the processes of amino acid activation and the subsequent transfer of the activated amino acids to the microsomal RNP particles may be similar in normal and malignant cells. The difference appears to lie in the rate at which newly formed proteins are transferred from the RNP particles to the soluble fraction of the cell. (Supported by CCNSC,N.C.I., NIH Contract #SA-43-ph-1886.)

6 *Biochemistry*

ENZYME SYNTHESIS IN E. COLI TREATED WITH 5-FLUOROURACIL. Jack Horowitz*, Jussi J. Saukkonen* and Erwin Chargaff. College of Physicians and Surgeons, Columbia University, New York City.

The formation of protein was examined in several strains of E.coli inhibited by the uracil analogue 5-fluorouracil which is known to interfere with nucleic acid metabolism. When a uracil-requiring mutant was exposed to the fluoropyrimidine in the absence of uracil it was found that, despite a doubling in the amount of total protein, no induction of β-galactosidase occurred nor did the enzyme activity increase in cells previously treated with an inducer. On the other hand, the activity of catalase and succinic dehydrogenase, two constitutive enzymes in this auxotroph, increased under these conditions. In strains of E. coli in which β-galactosidase is a constitutive enzyme 5-fluorouracil blocked a rise in the enzymic activity. The uracil analogue also greatly inhibited the induction of this enzyme in a wild strain of E. coli.This inhibition could be partially reversed by uracil but not by thymine. The induction of another enzyme, D-serine dehydrase, was also strongly inhibited by 5-fluorouracil. A more detailed study of some of the proteins formed in the presence of the fluoropyrimidine is in progress.

7 *Biochemistry*

MITOCHONDRIAL FORMATION OF AN RNA-LIKE MATERIAL. N.W. Penn (intr. by A. Novikoff). Dept. of Biochemistry, Albert Einstein Coll. of Med., Yeshiva University, New York.

The occurrence of nucleotide-peptide compounds in algae, yeast, bacteria and mammalian tissue has been reported by a number of investigators. In the present studies, this type of compound was found to be a product of cofactor-dependent breakdown of serum albumin added to a liver mitochondrial fraction. Consideration of the possible role of the nucleotide-peptide compounds in cellular metabolism suggested that acceptors for either or both of the nucleotide and peptide moieties might be present in the mitochondrial or other subcellular fractions. A material has been isolated from the mitochondrial fraction which becomes isotopically labeled on incubation with C^{14}-ATP. It is precipitated by ethanol in the presence of carrier RNA, and remains with the carrier through alcohol-ether extraction and reprecipitation. It is degraded by base and RNAase. A minimum of 80% of the total radioactivity is recovered as 2',3'-AMP on basic hydrolysis, and approximately 1.5% of the initial added isotope is found in the combined AMP and adenosine fractions. Formation of this RNA-like material is dependent on the addition either of serum albumin or amino acids.

8 *Biochemistry*

STUDIES ON THE NUCLEOTIDE REQUIREMENT IN THE TRANSFER OF AMINO ACIDS FROM SOLUBLE RNA TO MICROSOMAL PROTEINS. Kivie Moldave and Lois G. Grossi*, Dept. of Biochem., Tufts Univ. Sch. of Med., Boston, Mass.

Previous studies in this laboratory indicated that the transfer of RNA-bound amino acids to ribonucleoprotein particles requires GTP, ATP, an ATP-generating system, and a soluble cellular fraction obtained after precipitation of the pH 5-insoluble residue from rat liver supernatant. Dialysis of the pH 5-supernatant fraction resulted in a marked loss of activity which was recovered on recombination of the dialysate and the non-dialyzable component. A purified preparation of the non-dialyzable fraction has been obtained which is essentially nucleotide-free but contains 10% RNA; this activity, as reported previously, was not destroyed on heating at 100° for 10 min. at neutral pH. The dialyzable component as well as the ATP-generating system can be replaced by slightly higher levels of various nucleotides. Evidence will be presented which suggests that the function of added nucleotides or the ATP-generating system may be to generate GTP which is the only nucleotide required in this system.

9 *Biochemistry*

COMPARISON OF THE EFFECTS OF CHLORAMPHENICOL AND 8-AZAGUANINE ON BIOSYNTHESIS IN BACILLUS CEREUS. H. George Mandel and Robert L. Altman*. George Washington Univ., Washington, D.C.

Recent studies from this laboratory have revealed that the partial inhibition of growth of B. cereus by the purine analog, azaguanine, led to a profound depression of protein synthesis whereas RNA, DNA and cell wall synthesis continued. Since the structurally unrelated antibiotic, chloramphenicol, is known to produce similar biochemical effects in other bacteria, the relative responses of the two inhibitors were examined in B. cereus. Comparisons were made after corresponding increases in turbidity of the bacterial suspensions. The incorporation of methionine-S^{35} and lysine-C^{14} into protein, and protein synthesis were more sharply curtailed by chloramphenicol (10 ug/ml) than by azaguanine (15 ug/ml). Studies with diaminopimelic acid-H^3 and chloramphenicol suggested that the rate of cell wall synthesis was related to the slower growth rate whereas with azaguanine these effects were almost independent. Both drugs increased the utilization of labeled phosphate for RNA and DNA synthesis while only chloramphenicol readily produced a direct depressant effect on uracil uptake for pyrimidine nucleotide formation. Other shifts in the use of precursors for nucleic acid bases were also noted with this drug. Significant instability of RNA synthesized during inhibition of growth could not be shown.

221. RADIATIONS

1 *Physiology*

A MEASURE OF RESIDUAL RADIATION DAMAGE TO THE RAT ERYTHROCYTE PRECURSOR SYSTEM. Siegmund J. Baum (intr. by Edward L. Alpen) U. S. Naval Radiological Defense Lab., San Francisco, Calif.

Female rats were exposed every three months to 300 r of 250 KVP X Rays for four replications. On days 2, 3, 4, 5 and 6 after each irradiation the animals were injected with Fe^{59} and the maximum iron incorporation obtained. The Fe^{59} uptake was observed to be depressed to 14.5 per cent of control values in rats exposed to the first dose of 300 r. This was further reduced to 6.3 per cent after four radiation exposures. After injection on days 2 to 6 post-irradiation, maximum Fe^{59} incorporation increased exponentially. The recovery rate was similar for all four replications. Iron incorporation was doubled within 1.55 to 1.35 days and the fractional increase in maximum iron incorporation per day ranged from 0.447 to 0.513 for the four replications. Comparing the maximum iron incorporation on any given day from replication to replication, it was found to decrease exponentially. Assuming that the iron uptake was related to the restoration of erythrocyte bone marrow cellularity these data permit the calculation of an estimate of residual radiation damage to the erythrocyte precursor system. It is concluded that each repeated radiation exposure resulted in a residual injury to the erythrocyte precursor system which accumulated exponentially.

2 *Physiology*

EFFECTS OF X-IRRADIATION ON THE CHEMICAL COMPOSITION OF RABBIT BRAIN. G. R. Rowley and R. J. Dellenback (intr. by W. S. Root). College of Physicians and Surgeons, Columbia University, New York.

The heads of approx. 1 yr. old white rabbits (3.4-5.8 kg body wt.) anesthetized with Nembutal were X-irradiated with a total dose of 2000 r at a rate of 100 r/min. The control and test animals were kept in individual cages for 7 days after irradiation and the body wt. and neurological signs recorded. All animals were sacrificed on the 7th post-irradiation day, the brains removed, cleaned of blood, stripped of the pia mater and separated into 4 distinct regions: Gray matter, Midbrain including colliculi and hypothalamus, Medulla, Cerebellum. These tissues were individually analyzed for water, lipid, protein and undigested acid-insoluble residue and the results expressed as % of the initial fresh tissue wt. The Na and K content of each tissue was also determined and expressed as meq/kg of fresh tissue. No significant change in water content was found after head X-irradiation. A significant ($P < 0.05$) increase in the % lipid and protein was found in the midbrain and a significant decrease in the undigested acid-insoluble residue of the cerebellum was observed. The Na content of the cerebral gray metter showed a significant increase. The K content of all the regions studied showed no significant change from the control.

3 *Physiology*

ENHANCEMENT OF RADIOSTRONTIUM EXCRETION IN MAN. Herta Spencer, Arthur Feldstein and Joseph Samachson (intr. by Eugene Y. Berger.) Montefiore Hospital, New York.

Ionic calcium and NH_4Cl, administered singly or combined at the time of the injection of Sr^{85}, are effective in removing radiostrontium from man. NH_4Cl, given orally 2 weeks after intravenous administration of Sr^{85}, is still effective in raising its urinary excretion. In this "late" phase, intravenous calcium was tested in 5 and its combination with oral NH_4Cl in 3 patients under controlled dietary conditions. Infusions of ~470 mg Ca, given on days 15 to 17 of the study, led to increase of urinary Ca and Sr^{85} excretion, which was further raised by adding 9 gm NH_4Cl orally for 5 days. Single infusions of calcium gluconate were given to 20 patients on days 4-28 after the injection of Sr^{85}. The chelate di-aminoethylether tetraacetic acid, BAETA, Log K_{Ca} = 10.06, Log K_{Sr} = 9.36, was tested on Sr^{85} excretion in 5 patients. Ca-BAETA (4 gm) was infused on the day of injection of Sr^{85} and on 2 days thereafter. The urinary Sr^{85} excretion increased by 5.2, 14.2, 14.5 and 15.5% of the administered dose in 4 patients but was not higher in the 5th patient who had a high calciuria prior to the chelate infusion. The studies indicate that the degree of enhancement of Sr^{85} excretion depends on the baseline calcium excretion, irrespective of the enhancing agent used. (Supported by a grant from the U.S. Atomic Energy Commission.)

4 *Physiology*

RADIATION-INDUCED DEPRESSION OF DNA SYNTHESIS IN THE REGENERATING RAT LIVER. S. Okada and C.M. Wheeler (intro. by H.A. Blair). Univ. of Rochester, Rochester, N.Y.

Partially hepatectomized rats were given 800 r of x-rays to the regenerating liver at the pre-DNA synthetic period, and sacrificed during the DNA synthetic period. The activities of DNA synthesising enzyme system, deoxyribonuclease II, deoxyribose aldolase, thymidine phosphorylase, dihydrothymine dehydrogenase and hydrase have been studied. In the irradiated rats, the activity of DNA synthesizing enzyme system (Bollum and Potter, J. Biol. Chem. 233:478, 1959) was almost completely blocked, but the activities of the others were little different from that of non-irradiated rats. The possibility that radiation depresses DNA synthesis by damaging the DNA primer was also investigated. When calf thymus DNA and calf thymus nuclei were irradiated before being used as a primer for thymidine-H^3 incorporation in the Bollum and Potter system, no significant depression was observed of thymidine H^3 incorporation into DNA exposed to 20,000 r and into isolated nuclei exposed to 200,000 r. These results suggest that radiation does not affect DNA synthesis by damaging the template, thereby preventing replication but rather by depression of the DNA synthesizing enzyme system. Supported in part, by a grant from the U.S.A.E.C.

XUM

5 *Biochemistry*

RELATIONSHIP BETWEEN THE STRUCTURE AND PHOTOSENSITIZING ACTIVITY OF PSORALENS. M. A. Pathak* and J. H. Fellman*. (Intr. by W. R. Todd.) Univ. Oregon Med. School, Portland, Oregon.

Photosensitizing activity of psoralen and its derivatives was studied. In terms of erythemal response on mammalian skin in presence of UV light > 320 mu, psoralen and 4,5' and 8 methyl substituted psoralens were very active. Methyl substitution at 3 and 4' positions, alkyl substitution larger than methyl, substitution with methoxy, amino, hydroxy, nitro, acetyl, acetamino, bromo at 8 or 5 positions resulted in either partial or complete loss of activity. Oxazolocoumarin, isopsoralen or coumarin ring structures were inactive. Structure or functional group relationship necessary for photosensitization failed to emerge. The activating and the fluorescent wavelengths of biologically active and inactive furocoumarins were investigated. The regions of 265-280 mu and 340-380 mu were found to be associated with the active psoralens. The fluorescence peaks for the activating wavelengths were in the region of 420-460 mu. The inactive furocoumarins did not show these activating and fluorescent peaks. The action spectrum determined by the photosensitized destruction of lactic-dehydrogenase activity induced with psoralen was by 240, 290, 320 and 360 mu. This corresponds to the absorption spectrum of psoralen (245, 295 and 326)mu.

(Supported by grants from PHS #C-2837(C3) and B-1572(C 151).

6 *Biochemistry*

MODIFICATION OF YTTRIUM DISTRIBUTION BY CHELATING AGENTS IN MICE. Elsie Siegel and Herta Spencer (intr. by Emil J. Baumann.) Montefiore Hospital, New York.

The distribution and excretion of rare earth isotopes can be modified by chelating agents. A strong chelate, diethylenetriaminepentaacetic acid, Y-DTPA, Log K = 20.35 and a weaker chelate, N (2-hydroxyethyl) iminodiacetic acid, Y-HEIDA, were given intravenously at metal/chelate molar ratios ranging from 1:1 to 1:5. 0.03 mg and 0.001 mg Y^{+++} labeled with Y^{91} was used in chelated and in ionic form. The excretion of Y^{91}-DTPA was almost quantitative in 24 hours at both yttrium levels and at low or high molar ratios; the small percentage of Y^{91}-DTPA retained was distributed in all tissues. However, the different metal/chelate molar ratios resulted in differences in tissue distribution when 0.03 mg Y^{+++} was injected as Y^{91}-HEIDA. For instance, the Y^{91} concentration in liver was 56% at a metal/chelate molar ratio of 1:1.2, while it was 1/3 of this value at the 1:5 molar ratio, resulting in higher Y^{91} excretions and concentrations in kidney, muscle and bone. Y^{91} tissue distribution of 0.001 mg Y^{+++}, injected at different molar ratios, was similar to 0.03 mg Y^{+++} except for higher Y^{91} bone content with increasing molar ratios. The increased excretions and lower deposition in liver indicate that excess chelate increases the stability of relatively weak metal chelates. (Supported by a grant from the U.S. Atomic Energy Comm.)

7 *Pharmacology*

WHOLE BODY AUTORADIOGRAPHY OF C^{14}-IPRONIAZID IN MOUSE. V. Nair,* A. Hanngren,* H. Lal* and L. J. Roth. Dept. of Pharmacology, University of Chicago.

The metabolic fate of iproniazid in the mouse is studied utilizing the whole body autoradiography technique of Ullberg (Proc. 2nd Internat. Conf. Peaceful Uses Atomic Energy 24: Part I, 248, UN Geneva 1958). Drug was administered I.V. at intervals of 2, 10, and 20 min; 1 and 24 hrs. in doses of 50 mg/kg. Two minutes after injection the drug was found in various tissues including the skin and eye; those having concentrations higher than the blood were the stomach walls, gall bladder, kidney, urinary bladder and liver. After two minutes activity was found in the CNS, the hypophysis showing the highest concentration. After 10 min. the stomach, intestines, gall bladder, kidney and urinary bladder showed the greatest radioactivity. Radioactivity in the stomach, intestines, kidney and urinary bladder increased up to 1 hr. with respect to the blood while that in the liver slowly decreased until 1 hr. after injection when it approximated that of the blood, decreasing in all tissues to almost zero in 24 hrs. The chemical characterization of the agents contributing to the radioactivity is in progress. These studies bring out the phenomenon, not reported earlier, that the drug enters the CNS as early as 2 minutes after injection and is excreted into the stomach, gall bladder, kidney and urinary bladder and crosses the placental barrier.

8 *Pathology*

HISTOLOGICAL STUDIES OF POTENTIATION OF X-RAY EFFECT BY ACTINOMYCIN D ON SKIN OF NORMAL MICE. C. L. Maddock, B. Brown*, G. J. D'Angio* and C. Tedeschi.* Children's Cancer Res. Fndn., Boston, Mass.

A previous communication by D'Angio, Farber and Maddock (Rad., 73:175, 1959) documented potentiation of gross x-ray effects by Actinomycin D on skin of normal mice. To add histological confirmation to these findings, CAF/Jax mice were subjected to a single x-ray dose of 3000r or 1800r to the right hind leg, with appropriate shielding and a single dose of I. P. Actinomycin D at 75 γ/kg. These agents were used alone and in combination. At 4, 7, 10, 14, and 21 days, groups of mice in different categories were sacrificed. Identical pieces of skin were removed and studied, after Bouin's fixation, and hematoxylin-eosin staining. Actinomycin D alone produced no effect. Accentuation of changes in the combination group, as opposed to the x-ray group alone, was observed. This included increased intensity and extensiveness of x-ray damage, as well as its earlier appearance. Thus, combining Actinomycin D at a non-effective dose with x-ray enhances the latter's damaging effect vs. skin of normal mice.

223. RADIATIONS–Ionizing

1 *Physiology*

HOMOGRAFT-REACTIVE LARGE MONONUCLEAR LEUKOCYTES IN PERIPHERAL BLOOD AND PERITONEAL EXUDATES. Leonard J. Cole and Richard M. Garver.* U.S. Naval Radiol. Def. Lab., San Francisco, Calif.

Injection of whole blood or separated leukocytes from A or A strain mice (parental) into sublethally (500 r) X-irradiated (LxA) F_1 hybrids results in wasting, lymphoid tissue atrophy and delayed deaths (Cole et al. Transpl. Bull. 6: 429, 1959). Such cells also elicit the rejection of bone marrow homotransplants in irradiated mice. Further evidence for the immunological nature of these effects have been obtained. Injection of $1.4x10^6$ nucleated blood cells from hyperimmunized A-strain mice (immunized with LAF_1 spleen), results in 100% deaths in sublethally X-irradiated LAF_1 mice, with a mean survival time of 14 days; whereas, $2x10^6$ blood cells from non-immunized A-mice elicit 100% deaths, but with a 34-day survival time. Similar accelerated rejection of bone marrow grafts in lethally irradiated recipients occurs when blood from specifically immunized homologous donors is inoculated. Blood leukocyte reactivity is not annulled by pretreatment (24 hrs prior) of A-strain donors with 10 mg cortisone, which elicits a marked drop in mononuclear cell counts, and a relative increase in large cells (10μ diam. or greater). The killing effect of A-blood cells is not duplicated by injection of $.8x10^6$ peritoneal exudate granulocytes, but has been obtained with $2.5x10^6$ peritoneal exudate cells containing macrophages.

2 *Physiology*

LETHALITY OF UPPER BODY X-IRRADIATION. Carl L. Hansen, Jr.,* Sol M. Michaelson* and Joe W. Howland. Dept. of Radiation Biology, Univ. of Rochester School of Med. and Dent., Rochester, N.Y.

Exposure of large portions of animals to ionizing radiation results in responses which are distinct from those of whole body irradiation. Upper body irradiation of intact beagle dogs has been studied in order to characterize the biological response to this type of exposure. Adult beagles of both sexes were exposed, without anesthetization or other medication to 1000 KVP x-ray. The upper body, consisting of the head and thorax was exposed while the shielding of the lower portion of the body was accomplished by a specially designed and fabricated lead shield 8 cm thick. The median lethal dose of whole body x-radiation in this group of animals was determined to be between 225-260 r, MTD. Exposure of the upper body to 800 and 1000 r midline tissue dose did not produce lethality during an observation period of sixty days. Following exposure to 1500 r one of ten animals died on the 11th post-irradiation day. A fourth group of ten animals has been exposed to 2000 r with an indicated lethality of greater than 50%. It is concluded that lower body shielding significantly raises the lethal threshold, as well as the LD 50/30. (This paper is based on work performed under contract with the U. S. Atomic Energy Commission at the University of Rochester Atomic Energy Project).

3 **Physiology**

URINARY TAURINE EXCRETION PATTERNS IN THE RAT, RABBIT, GUINEA PIG AND DOG FOLLOWING EXPOSURE TO WHOLE BODY X-IRRADIATION. Charles R. Angel* and Thomas R. Noonan. Dept. of Radiation Biol. Univ. of Rochester School of Med. and Dent., Rochester, N.Y.

Taurine (2 amino ethane sulfonic acid) has been reported to be elevated in the urine of rats following exposure to whole body x-irradiation. The present study compares the urinary excretion pattern of taurine in the rat, rabbit, guinea pig and dog for several days prior to and for seven days after exposure to physically equivalent doses of x-irradiation. The rat shows a four fold increase in taurine on the first post-radiation day, with a return to normal level by the fourth day. The rabbit shows a two fold rise on the third post-irradiation day and approaches pre-irradiation values on the sixth day. The guinea pig shows no consistent elevation of taurine throughout the seven day period. The dog shows no elevation of taurine for the first four days post-radiation and then variable increases are observed on the fifth, sixth and seventh post-irradiation days. These results indicate that the urinary excretion of taurine after whole body x-irradiation is species dependent. (This paper is based on work performed under contract with the U.S. Atomic Energy Commission at the University of Rochester Atomic Energy Project.)

4 **Biochemistry**

EFFECTS OF GAMMA RADIATION ON RIBONUCLEASE. E. Slobodian, M. Fleisher and S. Rubenfeld (intr. by W. Troll), Dept. of Radiology, New York Univ.-Bellevue Medical Center.

Solutions of crystalline ribonuclease have been irradiated with gamma rays from a Co-60 source at doses up to 125,000 r. Samples showing various degrees of inactivation were chromatographed on columns of Amberlite CG-50, which separate pancreatic RNase into the two components A and B (Hirs, Moore and Stein, J.Biol.Chem.200,493, 1953). The irradiated samples showed a gradual decrease in the A peak with increase in dose, but no loss of B activity until over 1/2 of the A component disappeared. At a dose which reduced B to 1/2 of its initial amount, only 13% of the A component remained. This same effect was observed when RNase was treated with $Fe^{+2} + H_2O_2$ (OH· radicals), namely, that there was little or no loss of B, with a marked destruction of the A component. The ninhydrin patterns were similar to those of irradiated samples. These results constitute further evidence that OH· radicals are involved in the radiation inactivation of RNase. The observed difference in sensitivity between A and B is of particular import.

5 **Pharmacology**

EVALUATION OF FAT EMULSION IN THE ACUTE IRRADIATION SYNDROME IN RATS. Hugo C. Moeller and Jerome M. Vaeth (intr. by J. Alfred Rider). Depts. of Medicine and Radiology, Univ. Calif. School of Medicine, San Francisco, Calif.

This study was undertaken to determine whether intraperitoneal administration of a fat emulsion would provide protection against the effects of total body irradiation. Sprague-Dawley rats, 120 days old, were subjected to the $L.D._{50}$ (30 day) of total body irradiation. The animals were given 250 kv of x-irradiation with a half-value layer of 2.9 mm. Cu, distance 1 meter. The fat used was a 15% cottonseed oil emulsion. One group of rats received 1 gm. and a second group 2 gm. of the fat emulsion per Kg. body weight daily for 14 days following irradiation. In each experiment a control group of rats were given an equal volume of 5% dextrose in water. Mortality was definitely increased in the rats that received 2 gm. daily of the fat emulsion.

6 **Pathology**

FURTHER DOSE-RESPONSE STUDIES ON RADIATION-INDUCED MYELOID LEUKEMIA IN RF MICE. A. C. Upton and E. P. Sniffen.* Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee.

Male mice of the RF strain were exposed when 5-6 weeks of age to whole-body 250-kvp X radiation at a rate of 80-100 r/min (about 100 mice per group), as follows: group I, 0 r (control); group II, 25 r; group III, 50 r; group IV, 75 r; and group V, 100 r. The incidence of myeloid leukemia in the respective groups was: I, 4%; II, 6%; III, 10%; IV, 10%; and V, 17%. The median survival time (in months) of the groups was: I, 19; II, 19; III, 19; IV, 19; and V, 18. The incidence of lymphomas did not differ significantly from one group to another. These results are consistent with earlier observations suggesting that the incidence-doubling dose for myeloid leukemia in the RF mouse is less than 100 r and that the dose-response curve for this disease is not linear within the dose range 0-100 r.

7 **Pathology**

PROTEIN METABOLISM IN THE RAT-MOUSE CHIMERA. W. Friedberg and V. Jenkins (intr. by A. C. Upton). Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee.

The fractional rate of loss of I^{131}-labeled human serum albumin ($HSA\text{-}I^{131}$) is greater in X-irradiated (950 r) mice bearing transplanted rat bone marrow than in similarly irradiated mice protected with leg-shielding or in normal controls (Friedberg, International Journal of Radiation Biology, in press). Protein-bound radioactivity is not lost by renal excretion. Increased catabolism may account for the elevated protein loss in the chimera, but another possibility, leakage of protein through the wall of the gastrointestinal tract, is also under investigation. After intraperitoneal injection of $HSA\text{-}I^{131}$, small amounts of nondialyzable radioactivity are detected in the pooled 24-hour feces of both normal controls and chimeras, but neither the amounts nor the differences between the two groups appear significant. In studies with I^{131}-labeled polyvinylpyrrolidone ($PVP\text{-}I^{131}$), the feces of chimeras were more radioactive than those of normal control mice, suggesting increased permeability of the intestinal or gastric wall or loss via the bile duct. The significance of the $PVP\text{-}I^{131}$ results with respect to protein loss depends on the molecular size of the PVP in the feces and on the role of the liver in the loss of PVP and protein.

8 **Pathology**

DELAYED EFFECTS IN MICE IRRADIATED WITH PARTIAL BODY SHIELDING. G. E. Cosgrove* and A. C. Upton. Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee.

RF female mice were exposed at 12 weeks of age to 250-kvp X rays in the following groups (approximately 100 mice/group): I, 100 r whole body; II, 300 r whole body; III, 300 r upper third of body; IV, 300 r middle third of body; V, 300 r lower third of body; and VI, 0 r (controls). The median survival times were: group I, 17.7 months; II, 15.2 months; III, 19.6 months; IV, 18.1 months; V, 18.5 months; VI, 19.4 months. The thymic lymphoma incidence in the respective groups was: 25, 31, 11, 14, 18, and 18%. The myeloid leukemia incidence was 5, 19, 3, 3, 4, and 6%, respectively. The ovarian tumor incidence was 63, 56, 12, 41, 25, and 14%, respectively. These results support the concept that life shortening depends on a combination of radiation effects only some of which are induced by exposure of part of the body.

XUM

9 *Nutrition*

HEMATOLOGICAL RESPONSE IN SHEEP INDUCED BY BLOOD FROM IRRADIATED ANIMALS. José Souto and Donald L. Anderson (intr. by M. C. Bell.) UT-AEC Agric. Res. Lab., Oak Ridge, Tenn.

Circulating blood of donor sheep was deprived of leukocytes by exposing the animals to a Co^{60} gamma source for an acute whole body dose of 1600 r. The blood of normal sheep was replaced by that of the irradiated animals; during the substitution the total blood volume of each recipient was replaced twice. Under these conditions, animals having their blood replaced by that containing almost no leukocytes showed no decrease in total leukocyte counts. The number of granulocytes began to increase after about 2,000 ml. blood had been substituted, the increase involving both mature and young myeloid elements. Lymphocyte counts became normal after the first day post exsanguinotransfusion. In some recipients an increase in lymphocytes occurred between the second and twelfth week after the blood replacement. These changes were not observed in control recipients similarly treated by substituting normal for normal blood.

224. RADIATIONS–Isotope Techniques

1 *Biochemistry*

METABOLIC FATE OF LABELED CITRIC ACID. Thomas A. Cortese Jr.* and Adrian C. Kuyper. Wayne State University College of Medicine. Detroit, Michigan.

Citric acid is present in bones in a concentration of about 0.8 percent and in blood and liver in a concentration of about 2 mg. percent. In an attempt to measure the rate of exchange between blood citrate and that in the bones, radiocarbon labeled citrate was administered to rats and the rate of excretion of the labeled carbon as CO_2 was measured and compared with the rate of excretion of radiocarbon labeled acetate and succinate. Within 3 hours after intraperitoneal administration, the percent recovery of the injected radioactivity given as citrate-1,5-C^{14}, acetate-1-C^{14}, acetate-2-C^{14}, succinate-1-C^{14}, and succinate-2,3-C^{14} was 20, 74, 65, 89, and 60 respectively. After 30 hours the percent recovery was 36, 86, 88, 98, and 81 respectively. The relatively slow rate and incomplete metabolism of citrate as compared with these other acids is not due to the function of the citric acid cycle, but appears to be due to rapid exchange and fixation into the bone citrate. (Supported by Grant A-613 of the National Institutes of Health).

2 *Biochemistry*

STUDIES OF ISOTOPE EFFECTS WITH CARBONIC-ANHYDRASE USING C-14-BICARBONATE. Joseph L. Rabinowitz. Rad. Serv., VAH - and Biochem. Dept. Univ. of Penna., Phila., Penna.

Crystalline carbonic-anhydrase was prepared from the red corpuscles of sheep and human blood. These enzyme preparations were assayed for activity at various pH's, ionic strengths and temperatures. The assays were performed by the use of C-12 and C-14-bicarbonate. The recovered CO_2 was assayed by both gravimetrical and radioisotope techniques. It was shown that in all of these preparations a constant difference (Kc^{12}/Kc^{14} = ca 1.24) was observed in the rate of utilization of the substrate. The carbonic-anhydrase enzyme appears to prefer C-12-bicarbonate to C-14-bicarbonate. Carbonic-anhydrase was crystallized after acting on a substrate containing C-14 and was found to contain no radioactivity. This shows that no exchange of carbon occurred between the enzyme and the substrate during activity.

3 *Pharmacology*

PURIFICATION OF RANDOMLY LABELED TRITIATED BARBITAL. C. R. Boshart and G.K.W. Yim (intr. by T. S. Miya) Dept. Pharmacology, Purdue Univ., Lafayette, Ind.

Barbital exposed to 10 curies of H^3 gas for 2 weeks at room temperature and 0.39 atm. pressure, and equilibrated with two 30 ml. portions of water by the New England Nuclear Co. had a specific activity of 282 mc/g. Further exchange equilibration with six 10-15 ml. portions of water reduced the specific activity to about 266 mc/g. Purification was effected by preliminary extraction from an acidified aqueous solution with ether; recrystallization 3 times from toluene and once from water (specific activities were 70.4, 61.4, 70.8 and 69.8 ($\times 10^6$) cpm/mg., respectively); and column liquid-liquid partition chromatography. Analyses of column eluant fractions indicated a single peak of barbital with specific activity equal to that of the applied product. Paper chromatography yielded a single radioactive spot with R_f equal to unlabeled barbital. Biological stability was confirmed by isolation of barbital from rabbit serum 2 hours after IV injection. Specific activities before and after injection agreed within 5%. Final specific activity was 253 mc/g. (Supported by grant BF-8759, Public Health Service)

4 *Immunology*

TISSUE DISTRIBUTION OF RARE EARTH ISOTOPES IN MICE. Betty Rosoff, Elsie Siegel, Grady Williams and Herta Spencer (intr. by Valy Menkin.) Montefiore Hospital, New York.

Studies using radioactive rare earth isotopes have been motivated by interest in decontamination of fission products and possible use in cancer diagnosis and therapy. Previous studies indicated that distribution of rare earth metals can be modified by chelating agents. Therefore, the distribution of the rare earth isotopes Sc^{46}, Y^{91}, La^{140} and Sa^{153}, which represent a spectrum of ionic radii, was investigated. The intravenous injection to mice of these isotopes in ionic form resulted in very low excretion and high uptake in the liver. However, the intravenous injection of these rare earths, chelated with NTA or EDTA, changed the excretion and tissue distribution of these radioisotopes leading to greater uptake in bone and muscle. Also, the excretion increased in direct proportion to the stability constant of the rare earth chelate which was found to be inversely related to the ionic radius of the element. For example, the Sc^{46}-EDTA, which has an ionic radius of 0.68, is quantitatively excreted in 24 hours as compared to about 10% of the dose when La^{140}-EDTA is injected, which has an ionic radius of 1.06. The combination of certain rare earth radioisotopes and chelating agents can achieve selective tissue localization and radiation. (Supported by a grant from the U.S. Atomic Energy Commission.)

225. RADIATIONS–Protection

1 *Physiology*

LOW TEMPERATURE PROTECTION OF X-IRRADIATED MOUSE BONE MARROW CELLS. Menachem Lion* and L. H. Smith. Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tenn.

Mouse bone marrow cells exposed to 800 r in vitro are no longer able to promote recovery of lethally X-irradiated mice; 2-mercaptoethylguanidine, cysteine, cysteamine, or anoxia are protective to X-irradiated marrow cells (Smith, Exptl. Cell Research, 13: 627, 1957). Low-temperature protection of X-irradiated bacteria (Stapleton and Edington, Radiation Research, 5: 59, 1956) and yeast (Wood, Radiation Research, Suppl. 1, p. 332, 1959) has been demonstrated. In the present study, mouse bone marrow cells were X-irradiated in vitro at -196°C and their viability determined by their ability to promote survival in lethally X-irradiated mice. Isologous marrow cells, suspended in 15% glycerol in Tyrode's, were slow-frozen (1°C/min to -40°C), placed in liquid nitrogen, exposed to 800 r, rapidly thawed, and injected into mice exposed to 959 r. Up to 100% 30-day survival was obtained with ~10 X 10^6 marrow cells X-irradiated at -196°C, whereas none of the mice survived when given marrow X-irradiated at room temperature. Quantitation of this system remains to be investigated.

2 *Physiology*

REDUCTION IN INTESTINAL RADIATION DEATHS BY SINGLE POST-EXPOSURE INJECTION OF SUGARS OR CASEIN HYDROLYSATE. Marguerite N. Swift and S. Tom Taketa.* U.S. Naval Radiol. Lab., San Francisco, Calif.

A single I.P. injection of 15% glucose (3 ml/100 g body weight) given 1.5 hrs after supralethal whole body X-irradiation (990-1120 r) decreased the incidence of 3 to 5 day intestinal deaths in rats (24 hr pre-fasted animals). In a large series (150 rats), mortality was 31.1% in treated rats, 56.6% in controls ($p < 0.005$). This dose was similarly effective also when given at -2 hrs, ± 10 min or +4 hrs, but was ineffective at ± 24 hrs. A similar volume dose of isotonic 5% glucose at ± 1 hr was ineffective. To investigate the specificity of the protective action, the effects of fructose, galactose, xylose, sucrose and casein hydrolysate (CH), given at + 1.5 hrs in doses comparable to the 15% glucose, were tested. Except for sucrose, which appeared detrimental, all of these substances exerted a rather similar, or in the case of CH a greater, degree of protection. With the latter (80 rats): 22.5% mortality in treated rats, 77.5% in controls ($p < 0.001$). CH also afforded post-protection to some degree at a dosage of 150 mg/100 g, whether given in 15% or 5% solution. The physiological mechanism(s) responsible for protection with these various substances is undetermined at present. With regard to the sugars, a specific metabolic effect appears ruled out.

3 *Physiology*

THE EFFECT OF S-(2-AMINOETHYL)THIURONIUM BROMIDE HYDROBROMIDE (AET) ON THE SODIUM UPTAKE OF IRRADIATED HUMAN ERYTHROCYTES. Paul Novack and Bernard Shapiro (intr. by Robert M. Dowben.) Albert Einstein Medical Center, Southern Division, Phila., Pa.

Erythrocytes were washed and suspended in isotonic saline at a hematocrit between 5 and 10%. Tracer amounts of Na-22 were added and the suspensions were irradiated by a cobalt-60 source (8,000 r/hr.) and stored at 5° C. Erythrocyte Na-22 and Na-23 were determined at 24 hr. intervals. Results were related to hemoglobin content. Radiation resulted in an increased uptake of both Na-22 and Na-23. These results were similar to those reported by Sheppard and Beyl (J. Gen. Physiol., 34:691, 1951).who utilized doses of radiation over 6000 r. The addition of the radiation protective agent AET (10^{-2} M) prior to exposure diminished Na-22 and Na-23 uptake by both the irradiated cells and their unirradiated controls. The relative increase in sodium due to irradiation was not abolished but was significantly diminished by the AET over the dose range of 1250 to 4000 r. The mechanism of this action of AET has not been defined but is of obvious importance if related to its in vivo effect. (Supported by a grant from the NIH.)

4 *Biochemistry*

Effect of Continuous Antibiotic Feeding on X-Irradiation Mortality. W. H. Linkenheimer, H. Berger (intro. by E. L. R. Stokstad) Agricultural Division, American Cyanamid Co., Pearl River, N.Y.

Several reports have described the protective effect of antibiotics in acute radiation toxicity due to bacteremia. The effect of continuous Aureomycin feeding will be reported. Two groups of 200 mice each were exposed to 250 rad per week for three weeks. One group was fed Aureomycin at 20 ppm for the duration of the experiment; the other group was untreated. Although this irradiation dose should not produce acute radiotoxicity symptoms, a 40% mortality was observed during the first 2 weeks and was prevented by feeding 20 ppm Aureomycin. The acute symptoms were later demonstrated to be coincident with a Bartonella infection which is known to be Aureomycin sensitive. During the later stages of the experiment, that was 42 weeks in duration, no additional protective effect was suggested. A second and comparable experiment using Bartonella-free mice did not demonstrate the acute radiotoxicity effect with high mortality during the first two weeks as observed in the first experiment; however, the continuous feeding of Aureomycin at 20 ppm appeared to decrease mortality of the X-irradiated group during the early weeks of the experiment that was 60 weeks in duration.

5 *Biochemistry*

PROTECTIVE EFFECT OF AMINOALKYLISOTHIOUREAS, THEIR REARRANGEMENT PRODUCTS, MERCAPTOETHYL- AND PROPYL-AMINES AND THEIR DISULFIDES IN THE BONE MARROW SYSTEM. D. G. Doherty. Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee.

Bone marrow recovery in chemically protected, lethally irradiated mice was examined for the following series of compounds: 2-aminoethylisothiourea di HBr (AET), 2-aminothiazoline HBr, bis (2-guanidoethyl) disulfide di HBr, 3-aminopropylisothiourea di HBr (APT), 2-aminopenthiazoline HBr, bis (3-guanidopropyl) disulfide di HBr, 2-mercaptoethylamine (MEA), 3-mercaptopropyl amine, and bis (2-aminoethyl and 3-aminopropyl) disulfides. Most prompt recovery was obtained with AET followed closely by APT and MEA. The thiazolines and the disulfides were relatively ineffective in promoting bone marrow recovery. The results are in agreement with radiation mortality studies that show that the best protection at supralethal radiation dose levels is obtained with AET, APT, and MEA; the other compounds being ineffective over 1200 r.

6 *Biochemistry*

INFLUENCE OF COLLOIDAL STATE ON PLUTONIUM DISTRIBUTION AND ON EFFECTIVENESS OF DTPA THERAPY. A. Lindenbaum, J.F. Fried*, M.W. Rosenthal* and J. Schubert. Argonne Nat. Lab., Lemont, Ill.

The tissue distribution of Pu(IV) following daily treatment for 12 days with diethylenetriaminepentaacetic acid (DTPA) was studied in mice given Pu citrate via tail vein. Two nearly identical expts. were performed. In I, Pu given as a fresh soln. at pH ~5.5 was taken up nearly equally by bone (30%) and liver (34%) after 3 days. 12 days of DTPA therapy begun at that time reduced Pu in bone and liver to 55% and <3%, resp., of saline controls; liver reduction was mainly via the feces. In II, Pu given as a day-old soln. at pH ~6.0 was taken up less by bone (19%) and more by liver (46%) after 3 days; by the end of DTPA treatment Pu in bone and liver was 61% and 67%, resp., of controls. Differences in initial uptake of Pu and its subsequent removal after treatment are ascribed to differences in colloidal properties of the 2 Pu solns. This view is supported by data on Pu uptake by spleen prior to treatment (0.2% in I vs 1.3% in II) and by other tissue uptake studies using ionic and colloidal thorium. These results demonstrate the critical importance of colloidal state in tissue distribution and removal of easily hydrolyzable radioelements and are a part of continuing work on the assessment of long-term effects of therapy for highly toxic radioelements such as Pu. (Work done under auspices of U.S.A.E.C.)

7 *Pharmacology*

ANTAGONISM OF ROSE BENGAL INDUCED PHOTOSENSITIVITY. Keica Nishie* and Alexander C. Keyl. Department of Pharmacology, Northwestern University Med. School, Chicago, Illinois

The photodynamic effect of rose bengal in several biological systems, in vitro, has led to a number of alternative hypotheses concerning the basic mechanism of action. The fact that rose bengal, in vitro, in the presence of suitable reducing agents and oxygen, can produce inorganic free radicals identical with those formed in the radiolysis of water by ionizing radiation suggested the trial of a number of agents known to be protective against ionizing radiation such as x-rays, gamma rays, and neutrons in rose bengal sensitized mice subjected to sunlight and artificial illumination. It was found that cysteamine HCl(90 mg/kg), cysteine (925 mg/kg), S-2-aminoethyl isothiuronium (80 mg/kg), and dl-homocysteine (400 mg/kg) afforded significant protection from acute death when compared with controls subjected to three hours exposure to sunlight or equivalent artificial illumination. These results suggest that photosensitizing dyes, such as rose bengal, under proper conditions, produce effects in the biological system which are comparable with the secondary indirect effects of ionizing radiation. (Supported by P.H.S. Training Grant 2G-162)

XUM

8 *Pharmacology*

SOME QUANTITATIVE AND QUALITATIVE STUDIES WITH CELL-FREE RADIATION PROTECTIVE SPLEEN EXTRACTS. F. Ellinger, N. Henderson*, T. A. Strike*, B. F. Lindsley*, F. R. Henry*. Pharmacology Div., Naval Med. Res. Inst., Bethesda, Md.

Previous studies (Science 126: 1179, 1957; Atompraxis 4: 439, 1958) indicated radiation protection, without the late complications of "secondary disease," of homologous and heterologous cell-free spleen extracts, if given for 5 days after exposure of mice and guinea pigs to lethal doses of gamma Co^{60} radiation. This suggested a common chemical factor or factors in radiation protection. Quantitative studies revealed that a minimum of 360 mg equivalent of mouse spleen per injection protected mice and guinea pigs, while more than 1000 mg of guinea pig spleen were required to protect guinea pigs or mice. Protective preparations accelerate recovery of irradiated bone marrow and spleen and seem to reduce initial radiation leukopenia. Paper chromatographic analyses showed a higher content of taurine in mouse than in guinea pig extracts. This difference does not, however, account for the greater efficacy of mouse spleen, since 3 concentrations of taurine solution did not protect mice against radiation death. Neither is the total N nor NPN content indicative of radiation protectiveness. Quantitative relationships in amino acid contents appear more likely to be of importance.

9 *Pharmacology*

EFFECTS OF COMBINATIONS OF PROTECTIVE COMPOUNDS ON RADIATION INJURY. B. E. Hietbrink*, A. B. Raymund* and K. P. DuBois, U.S. Air Force Radiation Lab., Univ. of Chicago, Chicago, Ill.

This study was conducted to ascertain the ability of various radioprotective compounds given singly and in combination prior to 400r of x-ray to decrease radiation-induced changes in adenosine triphosphatase of spleen and thymus glands and acetylcholinesterase activity of the small intestine of rats. The protective effects were measured as per cent dose reduction based on a direct relationship between dose of radiation and magnitude of enzyme alterations. Thirty-day survival studies with normally lethal doses of x-ray were performed to correlate effects measured enzymatically with survival. Combination of 2-aminoethylisothiuronium (AET) (150 mg/kg) plus hydroxylamine (60 mg/kg) provided greater protection as measured enzymatically than either compound given singly and permitted 30-day survival of all of 10 rats given 850r and 6 of 10 given 1000r. AET alone prevented mortality of 2 of 5 rats and hydroxylamine failed to protect against lethality from 850 r. Similar enhancement of protection was obtained with the combination of hydroxylamine (75 mg/kg) with reduced glutathione (900 mg/kg) or cysteine (1000 mg/kg). The results of this study indicate that radioprotective agents with apparently different modes of action provide additive protection without additive toxic effects.

10 *Pharmacology*

THE BLOOD PRESSURE EFFECTS OF 3-AMINOPROPYL-N'-METHYLISOTHIURONIUM BROMIDE HYDROBROMIDE (APMT) IN THE CAT. Victor DiStefano, Phyllis S. Korn* and Daniel E. Leary*. Dept. of Pharmacology, Univ. of Rochester School of Medicine and Dentistry, Rochester, N. Y.

APMT is an antiradiation compound closely related in chemical structure to 2-aminoethylisothiuronium bromide hydrobromide (AET). Unlike other congeners of AET which are hypotensive agents, APMT in doses of 4 mg/kg produces in cats a moderate (15-20 mm Hg) but sustained (1/2-1 hour) hypertension which is unaffected by adrenolytic agents or by bilateral adrenalectomy. APMT (4 mg/kg) potentiates slightly the hypertension produced by epinephrine and nor-epinephrine. Evisceration and section of the spinal cord (C-1) also failed to prevent the hypertension induced by APMT. Spleen volume was increased by the administration of APMT. Plethysmographic measurements of hind limbs in cats revealed that APMT produces a biphasic response. An initial transitory increase in leg volume is followed by a slight but significant and persistent decrease which coincides with the hypertensive response. This study supports the hypothesis that APMT produces hypertension by the production of peripheral vasoconstriction.

11 *Pathology*

CHEMICAL PROTECTION AGAINST RADIATION DAMAGE. J. R. Maisin and D. G. Doherty (intr. by C. C. Congdon). Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee.

Protective effects of intraperitoneal S,2-aminoethylisothiourea (AET), aminopropyl-N'-methylisothiourea, and D-2-aminobutylisothiourea, mercaptoethylamine (MEA), serotonin, and AET and serotonin combined on the histology and mitotic activity in the intestinal crypt cells of X-rayed mice were studied. AET or MEA gave best protection; but all MEA-1500 r mice died by day 8. Oral AET or MEA also protected the intestinal tract. A combination of large doses of AET and small doses of serotonin was highly toxic. Four mg of AET orally protected the small intestine of 1500-r mice, though no mice lived past day 6; addition of 2 mg of serotonin gave better intestinal protection and longer survival. Optimal doses of AET and AET-serotonin gave the same protection at 1500 r. Mice exposed to 2500 r showed some intestinal recovery on the fifth day; with combined treatment, all mice died by day 6. Autoradiographs 5 min after i.p. injection of tritium-labeled AET showed diffuse labeling, higher in some organs than others. Labeling of the nuclei, cytoplasm, cell membranes, and mitotic figures was found. Various types of tumors contained labeled AET.

12 *Pathology*

PROTECTION FROM LETHAL X-IRRADIATION WITH VARYING DOSES AND AGES OF VIABLE HOMOLOGOUS AND ISOLOGOUS EMBRYO CELLS. N.S. Wolf (Intr. by E.T. Nishimura) Dept. Pathology, Northwestern U. Med. School, Chicago, Ill.

Adult mice were treated with LD 100 X-irradiation (810r) followed by intravenous injections of whole-embryo cells. It was shown that increasing doses of viable isologous embryo cells resulted in a corresponding increase of 30-day survival rate in treated mice. Increasing doses of viable homologous embryo protected the irradiated mice as effectively as the isologous cells at the lower dose levels. However, in comparing higher doses the homologous cells produced an inferior survival rate. Cells of extremely young embryos (11-12 days post-conception) were of little or no value in protecting adult mice receiving LD 100 X-irradiation. Older adult mice receiving lethal dose irradiation followed by intravenous injections of embryo cells had better 30-day survivals than similarly treated young adults. It is concluded that genetic relationship to recipient, viability, dose, and age of embryo cells, as well as the age of adult recipients, all influenced the survival rate among these mice receiving lethal radiation followed by whole embryo cells.

(Supported by PHS postdoct. fllwshp. #9343 & PHS grant C-3140 (C2).)

13 *Nutrition*

INFLUENCE OF AET UPON ERYTHROPOIESIS OF GAMMA-IRRADIATED RATS R. L. Preston and A. P. Raun (Intr. by W.H.Pfander) Aerospace Med. Lab., Wright Air Development Center, Dayton, Ohio

The action of AET against lethal effects of acute radiation is still to be shown. 4 expts were conducted to study the effect of AET on erythropoiesis in rats following acute gamma irradiation (Co-60). 8 rats/trt were used. Fe-59 was injected intraperitoneally 24 hrs following radiation and activity in 0.5 ml blood was detm 3 days later. Plasma activity was $< 1\%$ of the injected dose; thus plasma corrections were not made. Total RBC activity was calculated using 5.18 ml blood per 100 gm body wt. 400, 450, 600 and 625 r whole body radiation were used. AET (250 mg/kg body wt) injected intraperitoneally 15-20 min before radiation exposure did not alter the depression of Fe-59 uptake 1-4 days following radiation, compared to non-AET, radiated controls. AET alone had no effect upon Fe uptake. Rats kept for 31 days post-irradiation gave the following results for the non-protected and protected rats prior to 625 r (as % of controls): 1-4 days, 14.4 and 16.0; 8-11 days, 64.5 and 79.0; 15-18 days, 107.5 and 117.0; and 28-31 days, 95.5 and 113.0 respectively. Significance increased with each period ($P > .25, = .09, = .06, = .02$). Thus the initial effect of whole body irradiation upon erythropoiesis was not changed with AET; however, faster recovery occurred when animals had been injected with this material prior to irradiation.

226. RADIATIONS–Thermal

1 *Physiology*

PRODUCTION OF PAIN AND THERMAL BURNS IN SKIN AREAS PREVIOUSLY EXPOSED TO ULTRAVIOLET RADIATION. Alice M. Stoll, Leon C. Greene* and James D. Hardy. Aviation Medical Acceleration Laboratory, U. S. Naval Air Development Center, Johnsville, Pa.

To evaluate the relationship of tissue damage to pain sensation in areas of hyperalgesia, the skin of three subjects was irradiated with erythemal doses of ultraviolet radiation and then exposed to thermal radiation. Measurements were made of pain threshold lowering due to UV irradiation and, following the development of the hyperalgesia, of the lowering of blister threshold to thermal radiation. Pain threshold lowering was 4 to 7°C; blister threshold lowering was about 0.5°C, therefore, UV-irradiated skin is not more vulnerable to high temperature (50°C) burns than is normal skin despite the large pain threshold lowering. This finding is in keeping with the theory that pain is a function of the relative reaction rates at the thermal threshold and at skin temperatures above this threshold while damage depends upon the temporal integral of the damage rate. It also suggests the possibility of testing the efficacy of weak analgesics by use of areas of induced hyperalgesia.

2 *Physiology*

STUDIES IN THE KINETICS OF INFLAMMATION. E. Aschheim* and B. M. Zweifach. N.Y.U. College of Medicine, Dept. Pathology, N.Y.

Male albino rats were injected intravenously with I^{131} labeled human serum albumin (RISA) and then their abdominal skin subjected to heat injury or xylol inflammation. The increase of skin radioactivity was studied as a function of time. Data are presented in terms of clearance which is defined as the amount of blood completely cleared of radioactivity by a unit weight of dry skin. The increase in skin radioactivity due solely to the inflammatory process follows an exponential course during the first ten minutes, a straight line being obtained when the log activity is plotted against the log time in minutes. The relation found can be expressed by the formula

$C_i - C_c = a\ t^b$ where C_i and C_c are the clearances of the injured and control skins, t is the time in minutes, a and b are constants. A comparison was made of normal young and old rats and adrenalectomized animals also.
It is concluded that the accumulation of plasma proteins in the injured skin is due to a breakdown of a vascular barrier to diffusion.
This study was supported by a grant from the U. S. P. H. S. (2267).

3 *Pathology*

INHIBITORY AND ANTI-INHIBITORY FACTORS IN ACUTE AND HEALED BURN SERA BY TISSUE CULTURE TECHNIC. M.J. Rosenbaum*, L.F. Miller*, B. Sullivan* and S.R. Rosenthal. Naval Med. Research Unit #4, Great Lakes, Ill. and Univ. of Ill. College of Med., Chicago.

A technic is described for titrating substances in acute burn sera inhibitory to cell growth and for titrating anti-inhibitory properties of normal and healed burn sera. Varying quantities of acute burn sera were substituted for normal human serum normally used in growth of seeded HeLa cells in slanted tubes to titrate inhibitory effects on growth of the cell sheet. A box titration method was used to determine anti-inhibitory titers of normal and healed burned donor sera. Serial serum specimens taken early from severely burned children were inhibitory. Control sera did not cause inhibition. As recovery progressed inhibition diminished. Loss of inhibition was often first observed with serum collected several hours after transfusion with blood or plasma from a healed burned donor. Serum and gamma globulin from several recently healed severely burned donors had higher than normal anti-inhibitory titers. On the basis of preliminary observations, the technic is suggested as a useful *in vitro* method of employing viable cells to titrate and study the role of possible toxic-antitoxic humoral factors (in animals or man) in the pathogenesis and treatment of burns. (Aided by a grant from the Office of Naval Research (NR 114 161)

227. RADIATIONS–Tissue Effects

1 *Physiology*

THE RATE OF LENS OPACIFICATION IN X-RAY AND NEUTRON EXPOSED RATS. D. J. Kimeldorf and L. M. Cox*. U. S. Naval Radiological Defense Lab., San Francisco, Calif.

A colony of male rats was exposed to X Rays (450, 710 r) or neutrons (230, 320, 360 rads) at 3.4 months of age. Ophthalmoscopic examination commenced at 10 months of age and was continued periodically throughout the remaining life span. Some degree of opacification was present in all groups at the first examination. By the time the first case of complete opacification was observed at a given dose level ophthalmoscopy indicated the presence of lesser opacities in over 90% of the population exposed to the same dose. Cataract development in the rat was progressive with complete opacification observable at all dose levels. Only animals exposed to 450 r of X Rays failed to yield 100% incidence of complete opacification at 20 months. For a given age opacification was more advanced in neutron-exposed rats than in X-irradiated rats, although the neutron dose levels were roughly half those employed with X Rays. For each type of radiation the higher the dose the more rapid was the progression to complete opacification from the initial stage of cataract development. The average time for complete progression was 6 to 9 months for X Rays and 5 to 6 months for neutrons. An inverse relationship existed between dose and the age at which opacification was complete.

2 *Physiology*

THE INFLUENCE OF X-RADIATION ON A SERUM GLOBULIN COMPONENT IN THE C_3H_f/HeHa MOUSE. E.S.Goranson, E.A.Cinits* and E.A.McCulloch*. Depts. of Physiology and Medicine, U. of Toronto, and Div. of Biol. Research, Ont. Cancer Institute, Toronto.

It was previously reported (Fed. Proceed.18:57,1959) that exposure of C_3H_f/HeHa mice to single supra-lethal doses of whole-body X-radiation resulted 3 to 4 days later in a striking increase in a serum component with the electrophoretic mobility of an α-globulin. In mice protected by the injection of 10^7 isologous bone marrow cells, an elevated α-globulin was detectable by starch-gel electrophoresis months after irradiation indicating that the serum protein alteration is not a temporary effect of X-ray. Immunoelectrophoretic examination of the serum suggests that the elevated globulin is immunologically similar to a normal serum component. Glycoprotein extracted from serum of the irradiated mice exhibits a corresponding electrophoretic mobility and quantitative assays of glycoprotein show a 2 to 3 fold increase in this serum fraction following irradiation. To determine the possible source of the elevated glycoprotein following X-radiation chemical assays were carried out on bone marrow and on liver. The destroyed marrow in the irradiated animal contained negligible glycoprotein and the liver glycoprotein fraction was markedly reduced. The possibility of a more rapid production of glycoprotein by liver is being investigated.

3 *Biochemistry*

EFFECTS OF β-RADIATION ON PHOSPHORUS METABOLISM IN THE SKIN OF YOUNG RATS. I. A. Bernstein and P. Foster*, Univ. of Michigan, Ann Arbor, Michigan

Exposure of young rats (4-7 days old) to low levels of external β-radiation (about 20 rads) results in nearly complete mitotic inhibition in the cells of the epidermis and hair follicles of the skin for up to four hours post-radiation (Fukuyama, et al, J. Invest. Derm., 32, 39, 1959). Such dosages, however, do not appear to significantly alter the incorporation of intraperitoneally administered sodium phosphate-P^{32} into the nucleotides of either RNA or DNA isolated from the whole skin. At higher levels of exposure (e.g. about 50 rads), the absolute specific activities of the individual nucleotides of both nucleic acids are each reduced by 30-40 per cent. The specific activity of inorganic phosphate in the skin is not altered by this treatment. Since exposure to β-radiation can result in an immediate interference with mitosis in the skin while the incorporation of inorganic phosphate-P^{32} into the nucleic acids apparently continues at the normal rate, it seems possible that an aberration in a metabolic system other than DNA synthesis may be involved in this inhibition of cell division. Inasmuch as mitosis appears to be an "energy-requiring" process, the effect of low levels of β-radiation on the metabolism of ATP and other phosphate esters in the "acid soluble" fraction of the skin is being studied.

XUM

4 *Pharmacology*

EFFECT OF X-IRRADIATION ON THE DISTRIBUTION OF AN S^{35}-LABELED SULFONAMIDE IN MICE AND RATS. Mary F. Argus, Judith F. Kane and F.E.Ray (intr. by William P. Deichmann). Cancer Research Laboratory, University of Florida, Gainesville, Florida.

Previous studies (Argus, Hewson and Ray, Brit.J.Cancer,10: 321, 1956) on the distribution of radioactivity in the animal body, following intravenous injection of fluorene-2,7-di-(sulfonamido-2-naphthalene)-S^{35}, have revealed differences in the localization pattern in tumor-bearing animals as compared with tumor-free controls. To determine if this is a general stress phenomenon, young adult, male and female Wistar rats and CAF_1/Jax mice were exposed to a whole body x-irradiation of 150 r. A reduction was noted in the weight of the thymus and spleen, 48 hrs. following irradiation. Unlike the presence of a tumor, this irradiation dose did not affect the uptake of fluorene-2,7-di-(sulfonamido-2-naphthalene)-S^{35} by the liver and spleen. Significantly different localization of the S^{35}-labeled compound was found in the blood cells and thymus of irradiated as compared to non-irradiated male mice. (Supported by the Atomic Energy Commission and PHS Grant C-1356.)

5 *Pathology*

CYTIDINE LABELING OF ABNORMALLY ENLARGING CELLS. R. A. McGrath (intr. by C. C. Congdon). Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee.

Radiation dose-dependent nuclear enlargement of crypt cells in the mouse small intestine during the first 48 hours after 5000 r of X ray is not associated with increased total nuclear grain counts when tritiated thymidine is used as an indicator (McGrath, 1960, Intern. J. Radiation Biol., in press). In the present study, tritiated cytidine was injected into control and irradiated mice every 3 hours from time of X ray until killing at 48 hours. Autoradiograms were prepared. Grain counts per unit area were made over the largest crypt cells in controls and irradiated mice. The results show approximately equal grain counts per unit area in both cases. The grain count per unit area was lower in irradiated animals when tritiated thymidine was used. Since the volume of the largest crypt cells increases by a factor of about 10 after this X-ray dose, the evidence supports the conclusion that enlargement is a type of growth associated with an increase in cytidine labeling material in the cell.

6 *Pathology*

COMPARATIVE RADIOSENSITIVITY OF THE GASTROINTESTINAL TRACT Richard Thomason* and F.W. Jennings. U. of Chicago Path. Dept. Chicago, Ill.

Exteriorised segments of the G.I. tract of shielded albino rats were radiated. Gross and microscopic damage was compared at 20 days. Grossly, animals, except in stomach or duodenal radiation, were obstructed at the site of radiation with severe dilatation proximally. Small bowel obstruction was commonly severe enough to cause death. Though obstruction was seen in the radiated colon, animals usually survived. The stomach and duodenum showed necrosis of glandular epithelium and serosal inflammation and edema. The severity of necrosis became more intense with higher radiation doses, until ulceration of the mucosa was seen at 4000 r. The jejunum and ileum showed necrosis and dilatation at the lower doses of radiation. As the dosage was increased, the necrosis extended to include the muscularis and serosal fibrosis was seen. The colon showed necrosis and sloughing of the mucosa. While the degree of mucosal necrosis appeared to be similar at all levels of the G.I. tract following radiation, obstruction in both the jejunum and ileum lead to death. Muscularis necrosis and serosal scarring contributed significantly to the obstruction. This suggests particularly that radiation of the small bowel should be avoided.

7 *Pathology*

LIFE-SHORTENING FOLLOWING 800 r X-RADIATION TO THE PELVIS AND HIND EXTREMITIES OF RATS. J. J. Gambino*, L. R. Bennett, M. S. Billings*, and B. G. Lamson. Depts. of Radiology and Pathology, School of Med., Univ. of Calif., Los Angeles.

Life span duration of Long-Evans, female rats has been observed for 19 months after either a single exposure of 200 r x-radiation to the total body or 800 r exposure to the hind one-fourth of the body comprised of the pelvis, hind limbs, and tail. The 200 r total body exposure has reduced survival to 14 of an initial 41 rats (34.2%) alive at 19 months. The equivalent gram-roentgen exposure delivered only to the hind one-quarter portion of the body (800 r x 0.25) also has shortened life span, with 24 of 41 rats (58.6%) still alive in contrast to 36 of 47 alive (76.7%) in the non-irradiated controls. Shielding of the 75% of the body which includes the major thoracic and abdominal viscera has reduced but not eliminated the life shortening effects of external x-radiation applied to a portion of the body that does not contain organs known to be essential to life. (Supported by Contract No. AT-04-1 Gen. 12, between the Atomic Energy Commission and the University of California, Los Angeles, and in part under Contract No. AF 18(600)-1267, with the School of Aviation Medicine, United States Air Force, Randolph Air Force Base, Texas.)

8 *Pathology*

THE EFFECT OF DONOR AND HOST IRRADIATION ON THE RENAL HOMOGRAFT REACTION. C.F.Zukoski*, B.T.Jackson*, R.H.Egdahl, and D.M.Hume. Surg. Res. Lab. Med. Coll. Va., Richmond, Va.

Host whole body radiation permits successful bone marrow homotransplantation. The present studies were undertaken to determine whether TBR to donor or host would permit successful whole organ homotransplantation. Adult mongrel, previously splenectomized dogs were hosts for kidney homotransplantation. Either the donor or host was given whole body radiation a few hours prior to transplantation at doses of 600, 1000, 1200, and 1500r from a 1000 KVP Xray machine. Results indicated that all four doses of radiation to the donor animal failed to prevent the characteristic homograft reaction pattern or alter survival period. Radiation of the host with 600r or 1000r failed to prevent the homograft reaction in the transplant. Radiation of the host with 1200r prevented the usual transplant rejection changes in most animals for as long as they survived (4-13D), and with 1500r to the host no reactions were observed in any transplants. It is concluded from these studies that the cellular infiltration which occurs in canine kidney homografts is of host, not donor origin, and that 1200-1500r to the host is necessary in order to prevent these changes.

9 *Pathology*

MORTALITY AND HEMATOLOGIC RESPONSE IN RHESUS MONKEYS AFTER TOTAL BODY IRRADIATION AND INTRAVENOUS THERAPY WITH HOMOLOGOUS AND AUTOLOGOUS BONE MARROW. Richard J. McAlpine*, Jean K. Weston, Oscar J. Sorenson* and Joan Finzel*, Res. Div., Parke, Davis & Co., Detroit, Mich.

The experiments may be subdivided into two categories /A/ dose-mortality experiments (hematologic data obtained only at autopsy of moribund animals), and /B/ serial biopsy experiments (hematologic data obtained serially at various intervals throughout the post-irradiation period). Experiments in categories A and B involved 185 and 88 monkeys respectively. In category A experiments the dose of irradiation ranged from 450 to 650 r. Approximately half of the animals served as untreated controls and the remaining half were treated with pooled homologous bone marrow. Category A experiments indicated that slight enhancement of survival was achieved with homologous bone treatment after total body irradiation doses up to 600 r. However, this effect was unaccompanied by any evidence of enhanced hematologic recovery. In category B experiments the animals received doses of 500, 600, and 900 r followed by intravenous homologous or autologous therapy. Category B experiments also indicated that homologous bone marrow did not enhance hematologic recovery after lethal irradiation doses under the conditions of these experiments. However, autologous bone marrow was effective.

10 *Immunology*

COMPARATIVE EFFECTS OF FAST NEUTRONS AND X RAYS ON MARROW DNA CONTENT IN MICE. W. E. Davis, Jr., and L. J. Cole (intr. by M. S. Silverman.) U.S. Naval Def. Lab., San Francisco, Calif.

DNA analyses were made 2 to 24 hrs and 2 to 14 days following fast neutron and X-irradiation. Pairs of femurs were crushed in 0.001 M $CaCl_2$, the washed DNA-protein precipitate hydrolyzed with hot $HClO_4$ and DNA determined by the Dische method and from the density at 268 mμ. Time versus DNA content curves for neutrons (33 to 365 rad) and X-rays (45 to 810 rad) were characterized by early depletion (2 to 4 hrs), and by recovery - the extent and time depending on the dose. Recovery was first noted at 15, 24 and 48 hrs following 33, 66, and 139 rad of neutrons or 45, 150 and 225 rad of X-rays, respectively. With doses above 160 rad of neutrons or 400 rad of X-rays, a minimum DNA level of 300-400 μgm per femur pair (normal=1100 μgm) persisted until death or recovery occurred. Dose-response curves for neutrons and X-rays were similar (i.e., RBE=1) at 15 and 24 hrs. The dose giving 50% of maximum DNA depression (i.e., ED_{50}) was 20-40 rads at 15 hrs and 60-70 rads at 24 hrs. RBE values calculated from the respective X-ray versus neutron ED_{50} ratios were 130:65 (RBE=2.0) at 2 days; 165:80 rad (RBE=2.1) at 3 days; and 360:165 rad (RBE=2.7) at 6 days. The data suggest that the higher RBE of neutrons may be due to their greater effectiveness in delaying regeneration.

11 *Immunology*

INHIBITORY EFFECT OF SMALL DOSES OF X-RADIATION ON INCORPORATION OF IODODEOXYURIDINE (IDU) INTO DNA. S.L. Commerford*, D. Gitlin, and W.L. Hughes. Brookhaven National Laboratory, Upton, N.Y., and Dept. of Pediatrics at the Children's Hospital, Harvard Medical School, Boston, Mass.

When I^{131} labeled IDU, an analog of thymidine, was injected into mice in amounts of 0.1 μM or less, 8-10% was incorporated into DNA within 30 minutes. The remainder was rapidly degraded and excreted, largely as iodide, so that by 20 hours after injection the retained radioactivity in mice kept on stable iodide represented IDU incorporated into DNA. X-radiation was found to decrease IDU incorporation if irradiation preceded or immediately followed the injection of IDU. Marked inhibition appeared within an hour after irradiation while maximum inhibition was reached when IDU was injected 6-12 hours after irradiation. Recovery from this inhibitory effect was observed 24 or more hours after irradiation, the extent of recovery depending upon the size of the X-ray dose and the tissue. Exposure to X-rays later than 2 hours after IDU injection failed to inhibit IDU incorporation or to remove IDU already incorporated. Inhibition of IDU incorporation decreased as the X-ray dose decreased, but significant inhibition was observed after X-ray doses as low as 10 r or less than 2% of the LD_{50}. (Supported by U.S. Atomic Energy Commission and the National Institutes of Health.)

228. RENAL FUNCTION (KIDNEYS)

1 *Physiology*

RELEASE OF ADENOSINE COMPOUND INTO BLOOD STREAM BY ISCHEMIC KIDNEY. David B. Gordon. Univ. of Miami School of Med., Coral Gables, Fla.

In 1951 it was reported that the rabbit kidney, after 10 minutes of arterial occlusion, releases into the renal vein blood a substance which causes the opposite kidney to undergo blanching (Gordon and Flasher, Am. J. Physiol. 164:624, 1951). This substance is here identified as adenosine-5'-monophosphate (AMP). In 6 experiments in which blood was obtained which caused blanching of the opposite kidney, the plasma was analyzed by ion exchange chromatography using formic acid gradient elution on a column of Dowex-1-formate (Hurlbert, et al. J. Biol. Chem. 209:23, 1954). In every case a fraction was obtained consisting largely of AMP. Identification was based on (1) position in the elution series (2) ultraviolet spectrum and (3) paper chromatography. Analysis of arterial blood plasma showed AMP also present but in considerably lower amounts. Since AMP, when injected intravenously, causes the kidney to blanch, it is concluded that its secretion by one kidney after occlusion and release of the renal artery is the cause of the blanching of the other kidney. (Supported by grant #H-3681(C1) from the NIH.)

2 *Physiology*

ORIGIN OF URINARY HISTAMINE IN RATS. K.S. Kim (intr. by D.F. Magee). Univ. of Malaya in Singapore. Dept. of Physiol.

Urinary histamine is derived from three sources: diet, that synthesized in the body, and that released from body stores. Urinary histamine in fasting animals must be derived from the two latter sources. According to Wilson (1), intestinal bacteria probably contribute as much as half of the urinary histamine in rats but others (2) could not confirm his findings. This work was undertaken to clarify the confusion. The urinary histamine output was studied in normal fasting rats and in gastro-enterectomized rats with biliary fistulae. Histamine was estimated by bioassay. It was found that gastroenterectomy combined with biliary fistula did not reduce the urinary histamine output. Biliary fistula by itself does not effect histamine output in fasting rats. Therefore bacteria do not appear to contribute to urinary histamine in fasting rats. Similarly any other gut element is excluded as a major source.

1) Wilson, C.W.M. J. Physiol. 125:534, 1954.
2) Leitch, J.L.,V.G. Debley and T.J. Haley, Am.J. Physiol 187:307,1956.
Gustafsson, B., G. Kahlson and E. Rosengren
Acta physiol. scandinav. 41:217,1957.

3 *Physiology*

RENAL EXCRETION OF ENDOGENOUS METABOLITES IN MAN. THE GLYCINE EFFECT. Howard C. Elliott, Jr.* and H. V. Murdaugh, Jr. Depts. Chem. and Med., Univ. Alabama Center and Medical School, and VA Hospital, Birmingham, Alabama.

Excretion of hippuric acid has been studied in cases of renal disease using the theoretical interpretation of excretion data previously reported (Fed. Proc. 16:1957). In 21 cases of known renal disease it was found that there is a linear relationship between PAH clearance and K_E Hippurate. The average K_E in 25 normal controls was 2.7 hr^{-1} compared with an average of 1.2 hr^{-1} in subjects with renal disease. A transitory inhibition of endogenous uric acid excretion was noted following i.v. injection of 244 mg. of sodium benzoate. This inhibition could be reversed by glycine injection. Conversely, one gram of sodium benzoate caused increased uric acid excretion. Because of the similarity of these results to the "paradoxical" effects of varying doses of salicylate on uric acid excretion, the effect of glycine following salicylate administration was studied. A reduction of 29-45% in the rate of endogenous uric acid excretion after 0.5-1.5 gm oral salicylate can be reversed by administration of glycine. In summary: 1. a simple test of renal function is described, and 2. glycine is involved in the renal transport of uric acid.

4 *Physiology*

MECHANISM OF AMMONIA EXCRETION BY THE FISH GILL. Leon Goldstein* and Roy P. Forster. Dartmouth College, Hanover, N. H. and the Mt. Desert Island Biol. Lab., Salisbury Cove, Me.

Marine shorthorn sculpins, Myoxocephalus scorpius, were used to determine whether branchially excreted NH_3, which constitutes about four-fifths of the total nitrogenous wastes of fishes, is derived exclusively from diffused blood NH_3 or whether some is formed peripherally at the gill from plasma precursors. Measurements of cardiac output and NH_3 differences in blood entering and leaving gills of 500 gram fish disclosed that only 0.02 mmoles (14%) of the total 0.14 mmoles NH_3 excreted branchially per hr. could be accounted for as coming from preformed blood NH_3. Analysis of gill tissue homogenates showed that the total activity of glutaminase I and glutamic dehydrogenase in individual fish averaged 0.17 mmole NH_3/gills/hr. Thus, these two enzymatic mechanisms could account for all the NH_3 formed peripherally in the gill. Further support for this hypothesis was obtained from measurements of drops in non-protein acid-labile NH_3 as blood crossed the gill circulation. It is suggested that the "ammonotelic" nature of aquatic organisms generally could be explained by the activities of such peripherally located enzymatic mechanisms as are implicated in this study. (Supported by a grant from the National Heart Institute, H-4457.)

5 **Physiology**

HYPOSMOLAR URINE FORMATION DURING PLASMA HYPEROSMOLALITY. E. Bresler, J. Monroe, and J. Gilmartin (intr. by L. Churney.) VA Hosp. and Tulane Univ. School of Med., New Orleans, La.

The excretion of urines hyposmolar to contemporary plasma have been reported during osmotic diuresis induced with urea (Anslow and Wesson, Am. J. Phys. 180: 605 (1955). In the present study nembutal anesthetized dogs were infused with n-saline at the rate of 5 ml/min. They were then challenged with 150 ml 5 or 10% NaCl at the same rate of flow. Normal saline administration was then resumed. In this manner plasma concentrations ranging from 350 to 400 mos/l could be maintained for long periods with only slight change. During this time urines collected ranged from 50 to 150 mos less than contemporary plasma. In two instances exogenous pitressin was given but urines remained hyposmolar. In some instances serial urines were collected following the conventional stop flow procedure but without mannitol administration. A sharp decrease in osmolality appeared coincident with the sharp decrease in sodium and chloride concentrations in serial plots. The results indicate that under the extreme conditions induced in these experiments an intrinsic capacity of the kidney to produce dilute urine in spite of plasma hyperosmolality is revealed. This mechanism appears to be uninfluenced by exogenous pitressin. The locus for this process appears to coincide with that where active lowering of salt concentration takes place.

6 **Physiology**

AUTOREGULATION OF RENAL BLOOD FLOW RESULTING FROM RENAL DAMAGE. Jimmy B. Langston, Arthur C. Guyton and William J. Gillespie, Jr. (intro. by W. L. Williams.) Dept. of Physiology & Biophysics, Univ. Med. Center, Jackson, Miss.

A method has been devised for perfusing the kidney which makes it possible to measure the renal arterial blood flow while varying the renal arterial pressure from 0 mm. Hg to 300 mm. Hg and at the same time leaving the kidney's blood supply, lymphatics, and nerve supply completely intact. To date, this method has been used to determine over 100 renal pressure-flow curves, none of which have shown any evidence of autoregulation of renal blood flow. Each increase in pressure has been followed by an increase in renal blood flow, and as the perfusion pressure has been increased to higher and higher levels, each concomitant increase in blood flow has become progressively larger. An additional series of experiments has now been completed in which we first confirmed the above results and then damaged the kidney, either by subjecting it to a period of complete ischemia or by intentionally damaging the perirenal tissues. We then repeated the pressure-flow studies, and autoregulation of renal blood flow occurred in every instance. (Supported by a grant from the National Institutes of Health.)

7 **Physiology**

PRESSURE-FLOW-DISTENSION RELATIONSHIPS IN THE DOG KIDNEY. C. R. Replogle,* C. H. Wells* and W. D. Collings. Dept. of Physiology and Pharmacology, Michigan State University, East Lansing, Michigan.

The nature of distension dynamics in the dog kidney has been shown by Swann in studies with collodion-guaze wrapped kidneys, and by Hinshaw et al in studies on autoregulation. It has been possible to analyze distending forces in the kidney by measuring arterial pressure, venous pressure, blood flow, and distension, and by making analogies to other physical systems having the same mathematical relationships. By manipulating these parameters, the pressure-flow-distension relationships have been described for both static and dynamic situations in isolated kidneys. Plots of pressure-distension relations indicate three linear phases: the first, to about 14% distension, is interpreted as vascular filling; the second, at about 25%, may be interstitial filling; the last, is due to increased force necessary to stretch the kidney capsule. There appears to be a relationship between age of the dog and renal distension.

8 **Physiology**

RENAL MEDULLARY BLOOD FLOW AND ITS RELATION TO RENAL FUNCTION. K. Thurau, K. Kramer, P. Deetjen and H.Brechtelsbauer (intr.by Stephen Hajdu) Dept of Physiol., Goettingen Univ., Germany

Capillary passage time (t_p) and tissue-Hct. of renal medulla and cortex of anesthetized dogs were measured separately in vivo by photoelectric microunits. T_p was determined after rapid injection of T-1824 into the renal artery, tissue-Hct. by the method of Kramer et al.(Am.J.Physiol.165,229,1955). Renal perfusion-pressure was varied by means of extracorporeal perfusion technic; in other experiments water-diuresis was produced. Average t_p in the medulla was 30 sec., in the cortex 2.5 sec. Average medullary tissue-Hct. was 3%. Medullary blood flow (by Stewart-Hamilton formula) using an intravascular Hct. of 14% (Emery et al.,Lancet 7015,298,1958) was 21 ml./100 gm. tissue min.(cortical blood flow: 465/ml./100 gm. tissue min.). Unlike in the cortex, no evidence of autoregulation was found in the medulla. In diuresis experiments medullary blood flow increased with decreasing T^C_{H2O} and was not influenced by changes in free water clearance. It is concluded that (1) 'pressure-diuresis' is caused by increasing medullary blood flow which decreases the osmotic gradient from base to tip of the medulla, (2) changes in medullary blood flow observed during water diuresis result from alterations in viscosity and osmotic concentration of medullary blood in accordance with the counter-current theory.

9 **Physiology**

DISTRIBUTION OF TRANSIT TIMES IN DOG KIDNEY. Robert I. Macey, Zane Muhl and A. Omachi (intr. by A. V. Wolf) Univ. of Illinois College of Med., Chicago, Ill.

A procedure is presented for calculating the distribution of transit times, i.e., that portion of the total filtration which requires a given time to travel from the glomerulus to the site of urine collection. The method requires a knowledge of the time course of the urine and plasma concentration for a glomerular indicator during the transient state. The total filtration rate, mean transit time, and mean plasma concentration corresponding to a given urine sample can be calculated from this distribution of transit times. Experiments were undertaken involving the maintenance of a steady state concentration of plasma creatinine during a transient rise in the plasma inulin. The distribution of transit times was computed from the inulin data and total filtration rate compared with that measured by the standard steady state creatinine clearance. Preliminary results indicate reasonable agreement.

10 **Physiology**

CONCENTRATING ABILITY OF THE DISEASED KIDNEY, INFLUENCE OF GFR, SOLUTE EXCRETION AND HYDRATION. Neal S. Bricker, S.W. Kime, Jr.*, P.A.F. Morrin* and Tadeusz Orlowski* Washington University Medical School, St. Louis, Missouri.

Recent studies on dogs indicate that the concentrating ability of the chronically diseased kidney is not markedly impaired when the contralateral kidney is intact and the internal environment remains essentially normal. However diseased kidneys (D.K.) differed from intact kidneys (I.K.) in that a) during hydropenia maximum Uosm was somewhat lower and b) during osmotic diuresis T^CH_2O/GFR and Uosm were lower and Cosm/GFR and U_{Na} were higher. In the present studies the mechanisms of these differences have been investigated. Comparison of the hemi-infarcted kidney, which has a decreased but anatomically normal nephron population, with the contralateral IK revealed differences identical to those noted above. Moreover in the animals with unilateral disease, renal artery constriction of DK and aortic compression (proximal to both renal arteries) diminished all differences during osmotic diuresis. In hydropenic dogs subjected to aortic compression maximum Uosm of DK approached contralateral values. Prehydration markedly decreased T^CH_2O/GFR in both DK and IK. These results provide additional evidence that in dogs pathologic changes do not render the concentrating mechanism of the chronically diseased kidney impotent.

229. RENAL FUNCTION–Disorders

Biochemistry

11

AN INVESTIGATION OF THE NATURE AND STIMULUS OF "COMPENSATORY HYPERTROPHY" OF THE KIDNEY. David P. Simpson . (intr. by A. Baird Hastings.) Scripps Clinic and Research Foundation, La Jolla, Calif.

The P^{32} uptake in rat kidney DNA nucleotides was studied during compensatory renal hypertrophy. Forty-eight hours after unilateral nephrectomy and twelve hours after intraperitoneal administration of P^{32} as neutral phosphate, the P^{32} uptake was 1.8 to 4 times as great in the DNA nucleotides of the remaining kidneys as in those of controls. This is confirmatory evidence that the process of so-called "compensatory hypertrophy" involves an increase in cell number as well as in cell size and hence is more aptly termed compensatory hyperplasia.

A similar study was made on animals in which one ureter was drained into the peritoneal cavity so that urine from one kidney was reabsorbed into the circulation. There was no significant difference in the P^{32} uptake in renal DNA nucleotides compared to controls. This indicates that the stimulus for compensatory hypertrophy is some factor other than a dialyzable substance in the urine. (Supported by USPHS grant A-3574 (A)).

Biochemistry

1

ENZYME ADAPTATION IN POTASSIUM DEPLETION: PYRUVATE KINASE IN RENAL CORTEX AND MEDULLA. Robert P. Davis (intr. by H. A. Eder.) Univ. N. C. School of Med., Chapel Hill, N. C.

Physiologic abnormalities in renal function in K-deficiency may have associated defects in enzymes requiring K as cofactor. Both the concentration and the intrinsic-K dependent activity of pyruvate kinase (P.K.) were separately measured in kidney homogenates of K-deficient and pair-fed control rats. The deficient animals showed marked depletion of muscle and serum K, metabolic alkalosis and modest but significant depletion of kidney K, equal in both cortex and medulla. The ratio of DNA to tissue solids indicated true hyperplasia of the K-deficient kidney. Both concentration and activity of P.K. were markedly increased in K-deficient animals. Differences in measured P.K. activity due to K-depletion of the kidney cell itself were not significant in the face of increased enzyme concentration. Comparison of cortex and medulla showed that the entire increase in enzyme concentration occurred in cells of medulla, site of the anatomic and physiologic changes of K-depletion. DNA analyses indicated an increased quantity of enzyme per cell. Possible specificity of adaptive effects on P.K. in K-deficiency is suggested by the finding of an only modest increase in lactic acid dehydrogenase concentration, equivalent in cortex and medulla. (Supported by grants from USPHS and N.C. Heart Assn.)

Pharmacology

12

EFFECTS OF 2-4 DINITROPHENOL ON RENAL BINDING AND EXCRETION OF MERCURIAL COMPOUNDS. E.J. Cafruny and Charles Ross*. Univ. of Michigan Med. School, Ann Arbor, Michigan.

The major fraction of a dose of a mercurial diuretic is rapidly excreted in the urine. That portion which remains in the kidney is bound presumably as $R\text{-}Hg^{+}$ and/or Hg^{++} to sulfhydryl groups and possibly other active constituents of cellular proteins. Tubular excretion and fracture of a carbon-mercury bond are dependent on a source of energy. If phosphate-bond energy is utilized, 2,4-dinitrophenol (DNP) should influence both processes. Experiments were undertaken in an effort to test this hypothesis. Urinary excretion was studied in the dog; renal binding was determined in the rat. Mercuric ion was estimated colorimetrically following its liberation from organic complexes. DNP, in doses of 10-15 mg/kg, did not alter excretion of mercury. Renal binding of organic (meralluride) mercury was inhibited (from 259 to 150 ug/g of kidney) but binding of inorganic ($HgCl_2$) mercury was not affected. Tentatively, these data are interpreted as indicating that DNP depresses the mechanism which furnishes energy for liberation of mercuric ion from its attached organic moity. (Supported by a grant from the Office of Naval Research, Contract: NONR-1224 (26)).

Pathology

2

WALL/LUMEN FRACTION OF ARTERIOLES FOLLOWING BILATERAL NEPHRECTOMY AND URETERO-CAVAL ANASTOMOSIS. E. E. Muirhead and J. A. Stirman*. Wayne St. Univ. Coll. of Med., Detroit, Michigan.

The wall/lumen fraction (W/L) of arterioles is elevated following bilateral nephrectomy (dog) plus sustenance of life over 10 days by dialysis (Muirhead and Stirman in Pulmonary Circulation, Grune-Stratton, 1958). The arteriolar thickening seems due to hypertrophy and/or degeneration of media followed by fibrosis and hyalinization (arteriolarsclerosis). Renal transplantation seems to protect temporarily (Muirhead and Stirman, Surg.Gynec.& Obst. 103:673, 1956). The W/L was measured following bilateral nephrectomy plus dialysis and survival over 20 days (Group I), survival for 10-17 days (Group II), survival for 5-10 days (Group III; nephrectomy plus saline and sacrifice after 4 days [plus protein diet (Group IV); plus diet without protein (Group V); without diet (Group VI)]. All nephrectomized groups displayed prominent elevation of the arteriolar W/L (p vs normal < 0.001). Two types of distribution curves were obtained: one markedly different from normal (Groups I, II, IV) and one between the latter type and normal (Groups III, V, VI). W/L after uretero-caval anastomosis was not different from normal (p 0.9). The W/L was elevated rapidly and correlated with the hypertension. Intact renal tissue protected against arteriolar thickening as well as hypertension.

Pathology

13

EFFECT OF ADRENALECTOMY, CORTISONE AND HYPOPHYSECTOMY ON AMINONUCLEOSIDE NEPHROSIS IN RATS. Edwin R. Fisher and John Gruhn* Univ. of Pgh. and VA Hospital, Pittsburgh, Pa.

The effect of adrenalectomy and/or cortisone administration (0.5 mg./150 Gm. body wt.) and hypophysectomy on the biochemical features, morphologic appearance of the lesions including electron microscopy and histochemically demonstrable renal tubular enzymes-succinic dehydrogenase, and TPN and DPN diaphorases-attendant with the nephrotic state induced in rats with aminonucleoside (0.003 mg. Gm. body wt.) was investigated. The slight retention of NPN, moderate elevation of serum lipids, proteinuria, and accumulation of ascitic fluid were similar in adrenalectomized rats with or without supplementary cortisone administration; intact rats receiving cortisone, and hypophysectomized rats to that observed in rats treated with aminonucleoside only. A depletion of succinic dehydrogenase and DPN and TPN diaphorases and alteration of epithelial foot processes and basement membranes as revealed by electron microscopy were also similar in all rats experiencing aminonucleoside nephrosis. The former appeared related to the degree of proteinuria encountered. The administration of 1% saline as drinking water resulted only in an increase in ascitic fluid in all rats with aminonucleoside nephrosis regardless of treatment. These findings indicate that adrenal cortical function plays little primary role in the manifestations of aminonucleoside nephrosis.

Pathology

3

JUXTAGLOMERULAR CELL COUNTS AND HUMAN RENAL HYPERTENSION. Claire Turgeon* and Sheldon C. Sommers. Mass. Mem. Hosps. and Boston Univ. School of Med., Boston, Mass.

Using the Bowie stain, a new counting method assaying cells of 25 juxtaglomerular apparatuses has been employed to study surgical kidney specimens from normotensive and hypertensive persons. In unilateral renal disease of Goldblatt type cured by nephrectomy, a statistically significant hyperplasia of the juxtaglomerular cells was found. In analogous cases not cured by surgery, the juxtaglomerular cells tended to be more heavily granulated but not definitely hyperplastic. Correlations of the juxtaglomerular cell counts with a few renin assays of renal venous blood were unsuccessful, and corrected counts have been employed that included an estimation of the tubular epithelial Bowie-stained granules. These counting methods may assist in understanding the mechanism of the hypertension that occurs as a complication of certain renal parenchymal diseases like pyelonephritis.

4 *Immunology*

PATHOGENESIS OF EXPERIMENTAL CHRONIC PYELONEPHRITIS. R. Cotran*, E. Vivaldi*, D. Zangwill*, and E. H. Kass. Harvard Med. School and Boston City Hospital, Boston, Mass.

Experimental pyelonephritis, produced by intravascular injection of Gram-neg. bacteria, generally results in acute pyelo. with death of animal, or in spontaneous healing. When bacteriuria was produced in rats by injection of about 10^6 P. vulgaris into bladder, ascending pyelo. was produced consistently in absence of obstruction. After 70-130 days, majority of animals showed interstitial acute and chronic inflammation, tubular atrophy; focal dilatation, neutrophile casts, periglomerular fibrosis, but sparing of glomeruli. Animals with chronic pyelo. had statistically significant increase in systolic hypertension, and increased incidence of azotemia. Vesical and renal calculi often occurred. Thus, persistent bacteriuria leads to pyelonephritis in absence of obstruction, and brings about continuous reinfection of the kidney, thereby leading to chronic active pyelo. With persistent chronic pyelo., hypertension and azotemia may occur. (Aided by grants from the USPHS.)

230. RENAL FUNCTION–Diuresis

1 *Physiology*

CARBON DIOXIDE DIURESIS AT HIGH ALTITUDE. H. Valtin* and S.M.Tenney. Dept. of Physiology, Dartmouth Medical School, Hanover, N.H.

In normal, supine man the inspiration of 5% CO_2 in air leads to a diuresis mediated, presumably, by decreased secretion of ADH from the supraopticohypophysial system. Because of the well known interaction of hypoxia and CO_2 on the respiratory center, CO_2 diuresis was studied under hypoxic stress at an altitude of 14,246 ft. The same experiment, with two random, 30-minute exposures to 5% CO_2 in air, or its equivalent at high altitude, was performed on each of three subjects: a)at sea level; b)on acute exposure to high altitude; c)after 5-6 days' continuous residence at high altitude. All three subjects developed the expected water diuresis at sea level; none had a diuresis on acute exposure to high altitude; two developed a water diuresis of the same magnitude as at sea level, after 6 days' residence at altitude. The fact that arterial pH remained alkaline after prolonged exposure only in the one subject who failed to develop a diuresis, coupled with the fact that none of the three subjects developed the diuresis on acute exposure to high altitude, strongly suggests that a normal pH adjustment is essential to the development of CO_2 diuresis. This suggestion is consistent with the view that a decrease in intracellular pH may constitute the mechanism for CO_2 diuresis. (Supported by U.S.P.H. and N.S.F.)

2 *Physiology*

EVALUATION OF RENAL COUNTERCURRENT HYPOTHESIS IN APLODONTIA. W.C. Nungesser, E.W. Pfeiffer, D.A. Iverson and J.F. Wellerius (intr. by B. DeBoer.) Dept. Physiol. and Pharmacol. and Dept. Anatomy, U. of N. Dakota School of Med., Grand Forks, N. D.

Aplodontia rufa is a primitive rodent whose range is limited to the Pacific Northwest rainforest. Our object was to determine its renal concentrating capacity and to relate this capacity to the renal anatomy. Control balance studies were followed by progressive withdrawal of water by desiccating food. On a normal succulent diet the animal produced a daily urine volume equal to one-fourth to one-third its body weight. Maximal osmolar U/P ratios in the dehydrated animals were 2.0 to 2.4. Renal anatomical studies showed a primitive crest type kidney without papilla, a preponderance of cortical nephrons, few if any long looped nephrons, with no loops occurring in the thin segment, and no looped vasa recta. Aplodontia lacks the renal structural relationships for effective countercurrent multiplication and production of a highly concentrated urine. Lack of renal capacity for conservation of water may account for the species' limited distribution.

3 *Physiology*

UPTAKE OF RADIOMERCURY BY NEPHRIDIA OF PHASCOLOSOMA GOULDI. Roger L. Greif. Dept. of Physiol., Cornell U. Med. Coll, New York City and Marine Biological Laboratory, Woods Hole, Mass.

The excised nephridia of this marine invertebrate accumulate far more Hg^{203} from a solution of labelled chlormerodrin than do its other tissues. An artificial sea water suspending medium was used for these studies, buffered with .02 M TRIS to pH 8. To test reproducibility of the method, a mince of nephridia was shaken at 20°C for 150 minutes in suspending medium containing 5 micrograms of chlormerodrin per ml. of solution. 12 separate aliquots of tissue were removed, blotted, weighed, and counted. The mercury content was 54.7±5.1 micrograms per gram wet weight of tissue. Between 10° and 40°C, Q_{10} values for mercury accumulation were between 1.5 and 2.2. Radiomercury uptake by the nephridial mince at 20°C was inhibited approximately 60% by 10^{-3}M cyanide, penicillin G, and a cyanine dye. Less inhibition was produced by 10^{-3}M benemid. No inhibition was produced by 10^{-3}M 2,4 DNP, PAH, azide, iodoacetate, tetraethyl-ammonium-bromide or n-methyl-nicotinamide. The data suggest that the uptake of mercury by this tissue is a process requiring metabolic work, and may involve renal transport mechanisms found in mammals.
(Supported by a grant from the U.S.P.H.S.)

4 *Physiology*

FREE WATER FORMATION IN NORMAL DOGS. E. Lovell Becker. Dept. of Medicine, Cornell Univ. Med. College, New York City.

Free water is considered to be that moiety of urine which is osmotically unobligated and is produced by selective solute reabsorption in the distal tubule in the absence of antidiuretic hormone. This virtual volume is calculated from the urine flow minus the osmolal clearance. Unanesthetized normal dogs were studied four times with different osmotic agents to determine if a maximal rate of "free water" production could be demonstrated. Water diuresis was instituted in fasted animals with an oral load of 75 ml/kg. of body weight and maintained with an intravenous infusion of 3% glucose in water delivered at 18 ml/min. After three control periods at a constant urine flow, a second infusion containing either mannitol, urea, sodium sulfate, or sodium chloride was given at 20 ml/min. In all studies the regression line of a plot of the osmolal clearance against urine flow had a slope of less than one, and a maximal free water clearance could not be demonstrated. A number of variables may contribute to the observed rise in free water clearance during combined water and osmotic diuresis. These include increased reabsorbable solute presented to the distal tubule, lowering of the interstitial solute concentration in the renal medulla, and diminished back diffusion of water from the tubular urine.

5 *Physiology*

EFFECT OF DIURESIS ON FLOW AND COMPOSITION OF RENAL LYMPH. S. J. LeBrie* and H. S. Mayerson. School of Medicine, Tulane University, New Orleans 18, La.

Flow, protein and electrolyte concentrations of renal capsular lymph were followed before, during and after infusion of diuretic doses of mannitol, a mercurial diuretic (Mercaptomerin) and urea in Nembutalized dogs. Lymph flow generally increased in proportion to the extent of diuresis. Mannitol consistently produced a significant diuresis (average = 5 ml/min./kidney) which was accompanied by an average increase of 608 p.c. in lymph flow (range = 25 to + 2490 p.c.). Mercaptomerin produced variable and less consistent changes in urine and lymph flows. Preliminary results with urea suggest a similar correlation between lymph and urine flows. In spite of increased lymph flows, lymph protein concentrations frequently increased. Lymph electrolyte concentrations did not change significantly. These results are consistent with data previously obtained indicating that high renal vein pressures produce a marked increase in renal lymph flow and its protein concentration. We likewise ascribe the present results to increased hydrostatic pressure and protein filtration resulting from mechanical compression of renal blood vessels produced by increased tubular and interstitial pressures in the relatively rigid capsule. (Aided by grants from Office of Surgeon General, Dept. of Army and U.S. Public Health Service)

6 **Physiology**

RENAL MEDULLARY BLOOD FLOW. Lawrence S. Lilienfield, Herman C. Maganzini* and Mark H. Bauer.* Cardiovascular Research Laboratory, Department of Medicine, and Department of Physiology and Biophysics, Georgetown University Medical Center, Washington, D. C.

Recent attention has been focused on the renal medulla because of its high osmolarity. The role of the circulation in the regulation of this osmolarity is unknown. As a first approach to this problem, a method has been devised for measurement of plasma flow in the renal papilla. The principle used involves the measurement of the accumulation rate of I^{131} albumin in various regions of the kidney. This should be dependent upon the plasma flow to the region. Studies in 15 hydropenic mongrel dogs with U/P osmolal ratios of 3-4/1 as controls, revealed an average perfusion rate equivalent to 20 ml. of arterial plasma per 100 Gm. of renal papilla per minute. Under saline diuresis the papillary perfusion rate increased approximately two fold over the controls. Under osmotic diuresis with mannitol, the papillary perfusion rate was found to be less than one half that of the control group. These data suggest that the plasma perfusion rate of the renal papilla varies with induced alterations in urinary solute and water relationships and that hitherto unsuspected vascular mechanisms may be operating.

7 **Pharmacology**

ELECTROLYTE PATTERNS IN CIRRHOTIC AND CARDIAC PATIENTS AFTER FLUMETHIAZIDE. F. Steigmann, A. Dubin* and S. Alvarez*. Hektoen Institute for Medical Research of Cook County Hospital, Chicago, Illinois.

Flumethiazide (6-trifluoromethyl-7-sulfamyl-1, 4, 2-benzothiadiazine-1, 1-dioxide) was noted to induce a diuresis qualitatively similar to that of chlorothiazide, with less Na, and much less K loss and to maintain cardiac patients comfortably, without electrolyte imbalance. We compared 10 cirrhotic and 10 cardiac patients after Flumethiazide therapy. Urinary electrolytes were determined for two control days. Then the patients received 1,000 mg. of Flumethiazide b.i.d. The weight, the urinary volume and electrolytes were determined daily. The serum electrolytes were determined at the start and at the end of the observation period. Flumethiazide produced good diuresis in both groups of patients with marked output of Na and Cl and much less of K. Weight loss was more marked in cardiac patients. One cirrhotic showed signs of hypokalemia (K 2.9). One cirrhotic patient received Flumethiazide 2,000 mg./d. for several weeks without side effects. These results indicate that Flumethiazide is a useful diuretic in cirrhotic and cardiac patients.

8 **Pharmacology**

INHIBITION OF MERCURIAL DIURESIS BY NON-DIURETIC MERCURIALS. Tracy B. Miller* and Alfred E. Farah. SUNY, Upstate Medical Center, Syracuse, N. Y.

Non-diuretic organomercurials have been administered in various doses to anesthetized dogs to study the effects they might have upon the action of diuretic compounds. Intravenous administration of p-chloromercuribenzoate and p-chloromercuriphenyl sulfonate, non-diuretic compounds which are potent inhibitors of sulfhydryl dependent enzymes in vitro, prevented and also reversed the diuretic action of mersalyl, mercaptomerin, and mercuric chloride-glutathione complex. Ethyl- and methyl mercury chloride failed to inhibit mercurial diuresis. The observed inhibition occurs with minimal changes in glomerular filtration rate and effective renal plasma flow. Preliminary experiments indicate that chlorothiazide and sodium chloride diuresis are not affected by the inhibitor compounds. The inhibition is most likely due to a competition at the receptor sites between inhibitor and the diuretic mercurial compound. (Supported by a grant(No. H-2835(C3) from the National Heart Institute, PHS).

9 **Pharmacology**

DIURETIC EFFECT OF SOME 2- AND 3- SUBSTITUTED ANALOGUES OF HYDROCHLOROTHIAZIDE. K. Hwang, H. K. Iwamoto, L. Coen* and H. E. Johnson*. Dept. of Pharmacology, Abbott Labs., North Chicago, Illinois.

A group of 3- substituted analogues of hydrochlorothiazide (HC) has been studied regarding the diuretic effect in the rat by a modified method of Lipschitz et al. The potency was decreased for most of the compounds. 2-Methyl substituted HC, however, showed about twice the activity of HC. When 2-methyl substitution was added to a number of 3- substituted compounds, the diuretic activity was, in general, greatly improved over the corresponding mono-substituted compounds at the 3- position. Two of these compounds were of special interest, namely: 2-methyl-3-carbomethoxy HC and 2-methyl-3-chloromethyl HC. Both compounds, as well as other analogues, produced diuresis with an electrolyte excretion pattern similar to HC. Their potency ratios over HC were about 10 and 20, respectively. Anti-carbonic anhydrase activities of these two compounds and HC were about comparable. When doses of these two drugs producing maximal renal responses were combined with maximal doses of acetazolamide, additive effects were obtained. When these were combined with maximal doses of HC or chlorothiazide, no increase of the response was observed. These findings suggest a similar mode and site of action for all these chlorothiazide analogues.

10 **Pharmacology**

FURTHER PHARMACOLOGICAL STUDIES WITH BENZTHIAZIDE IN DOGS. A. Scriabine*, S. Y. P'an, D. Rowland*, and C. Bertrand*, Research Laboratories, Chas. Pfizer & Co., Inc., Groton, Conn.

Benzthiazide (3-benzylthiomethyl-6-chloro-7-sulfamyl-1,2, 4-benzothiadiazine-1,1 dioxide), a potent non-mercurial diuretic was further evaluated in dogs. Benzthiazide produced the maximal saluretic effect during the second or the third hour after oral (0.125 and 2.0 mg/kg) and within the first 10 to 30 minutes after i.v. administration (0.4 mg/kg). There was still a pronounced saluresis during the 6th hour after 2 mg/kg of the drug orally. The effectiveness of benzthiazide (1 mg/kg/day i.v.) daily for 14 days was demonstrated in six dogs which received 30 ml/kg of 0.9% NaCl orally. The urinary volumes and electrolytes excretion decreased during continued therapy, but were higher than on the control days before and especially after cessation of the therapy. Further renal clearance studies with benzthiazide indicated that maximal natriuretic and chloruretic effects were reached at 0.1 mg/kg whereas potassium and bicarbonate excretions were further increased at 1.0 mg/kg dose level. Benzthiazide increased sodium and chloride excretion in experimental alkalosis and acidosis. Stop-flow experiments were made to localize the natriuretic and kaliuretic effects of benzthiazide in the renal tubuli.

11 **Pharmacology**

DIURETIC RESPONSES TO NAQUA, A NEW BENZOTHIADIAZINE DERIVATIVE, IN CASES OF CONGESTIVE HEART FAILURE. Duncan E. Hutcheon and Teruo Takasu*. Depts. of Pharmacology and Medicine, Seton Hall College of Medicine, Jersey City, N.J.

A double-blind study was carried out to find the dose response relationships of Naqua, 3-dichloromethyl-6-chloro-7-sulfamyl-3,4-dihydro-1,2,4-benzothiadiazine-1,1-dioxide, when given orally to cases with chronic heart failure. Significant diuretic responses were observed following oral doses of 2, 4 and 8 mg. twice daily over a period of one week. The mean weight loss with standard deviation in 9 patients treated with 8 mg. twice daily for 7 days was 3.2±2.7 lb. No significant changes in serum creatinine concentrations were observed after doses as high as 8 mg. twice daily for 7 days and serum Na, K, Cl and CO_2 combining power remained within normal limits after the week of therapy. Similar diuretic effects were produced by 4 and 8 mg. Naqua and by 75 mg. hydrochlorothiazide in this study.

XUM

12 *Pharmacology*

RENAL GLUCOSE METABOLISM AND ITS RELATION TO DIURESIS. W.P. McCann. Dept of Pharmacology, Univ. of Colorado School of Medicine, Denver, Colorado.

Studies are in progress which attempt to assess the role of renal glucose metabolism in renal function. Initial renal arteriovenous difference studies in 3 unanesthetized dogs suggest that near the peak of diuresis after the rapid IV infusion of .6% NaCl, the kidney may extract glucose from the blood, while it generally puts glucose into the blood in the non-diuresing periods (for techniques, see McCann and Jude, Bull. Johns Hopkins Hosp., 103: 77, 1958). Studies of rat kidney slices in serum under O_2-CO_2 mixtures indicate that cortical tissues produce glucose, while medullary tissues rapidly use it. Glycogen changes appear to play but a small role in the in vitro observations. Renal glucose production may be related to normal renal function. Conceivably, glucose produced by proximal convolutions could be delivered directly to more distal segments for their use, via the intra-renal circulation, thus sparing peripheral glucose. These observations may also bear on the water intolerance of adrenal insufficiency (corrected by cortisone, but not by DOCA). (Supported by a Senior Research Fellowship SF-214-C, from the U.S. Public Health Service).

13 *Pharmacology*

PHARMACOLOGY OF 3-BENZYL-7-SULFAMYL-6-TRIFLUOROMETHYL-2H-3,4-DIHYDRO-1,2,4-BENZOTHIADIAZINE-1,1-DIOXIDE (BL-H368), A NEW, POTENT DIURETIC AGENT. D. E. Tisch, J. B. Hoekstra and M. H. Pindell (intr. by H. L. Dickison) Research Division, Bristol Laboratories, Syracuse, New York.

A large series of novel benzothiadiazine compounds have been screened for oral diuretic activity in unanesthetized dogs. BL-H368, the 3-benzyl derivative of hydroflumethiazide, was found to be more than ten times more potent than hydroflumethiazide itself and more than 100 times more active than chlorothiazide. Natruresis and chloruresis equivalent to that following other benzothiadiazine diuretics occurred but less potassium excretion was evident with BL-H368, an observation previously made experimentally with hydroflumethiazide and confirmed clinically; apparently a corollary of substitution of the trifluoromethyl group for chlorine in these compounds. Intravenous administration of 0.1 mg/kg of BL-H368 markedly depressed tubular reabsorption of sodium without altering glomerular filtration rate. The compound possesses less carbonic anhydrase inhibitory activity than chlorothiazide. BL-H368 has negligible acute toxicity orally (>10,000 mg/kg) and was well tolerated by both rats and dogs when administered daily for several months in enormous amounts relative to the effective diuretic dose.

14 *Pharmacology*

DISTRIBUTION STUDIES OF TRITIUM LABELED HYDROCHLOROTHIAZIDE IN MAN. Morton S. Comer* and Benjamin Calesnick. Laboratory of Human Pharmacology, Hahnemann Medical College, Philadelphia, Pa.

Single trace doses of tritium labeled hydrochlorothiazide, were administered orally or intravenously to patients with hypertension or cardiac failure. Blood and urine samples were collected hourly for the first 6 hours and at 12, 24, 48 and 72 hours. Studies revealed a double peak in both the plasma and urinary excretion pattern. Orally the urinary peaks occurred at 2 and 4 hours. Intravenously the urinary peaks occurred at 0.5 and 1.5 hours and the plasma peaks occurred at 1 and 30 minutes. The data of H. Sheppard et al (Fed. Proc. 18: 444, 1959) in rats, showed that the kidney and liver have the highest concentration of tritium after a dose of the drug. Correlating our data with theirs suggests a renal or extrarenal redistribution of this agent regardless of route of administration. The 24 hour urinary excretion of tritium was between 43 and 53% of the administered dose, which approximates the excretion rate of hydrated rats who received 0.16 to 0.31 mg./kg. of tritium labeled hydrochlorothiazide (H. Sheppard et al Fed. Proc. 18: 444, 1959).

15 *Pharmacology*

PHARMACOLOGY OF A NEW BENZOTHIADIAZINE DIURETIC. Richard M. Taylor*, Jeanne S. Mershon* and Martin M. Winbury. Schering Corp., Bloomfield, N. J.

Our studies of substituted benzothiadiazines have been directed toward compounds with marked diuretic and saluretic activity but possessing less kaluretic action than known agents in this series. Of the compounds tested, 6-chloro-3-dichloromethyl-3,4-dihydro-7-sulfamyl-1,2,4-benzothiadiazine-1,1-dioxide (DCH) was found to be the most potent derivative on a dose basis. It is approximately 10 times more active than hydrochlorothiazide and 50 to 100 times chlorothiazide in promoting the excretion of Na and Cl after oral administration to rats and dogs. In dogs, the Na/K ratio of the urine was found to be greater with DCH than with either of the other agents. Dose-response curves for Na excretion in dogs have demonstrated that the minimally effective oral dose is 0.002 mg/kg and that maximum responses are achieved at doses as low as 0.1 mg/kg. In renal hypertensive dogs DCH produced a significant reduction in blood pressure but had little or no effect in normotensive dogs. Preliminary experiments also suggest that DCH, unlike hydrochlorothiazide and chlorothiazide, is not eliminated by tubular secretion. These experiments will be discussed in relation to the duration of action of hydrochlorothiazide and DCH.

16 *Pharmacology*

A METHYL-SULFONE DERIVATIVE (G-34764) OF PHENYLBUTAZONE WITH PROLONGED URICOSURIC EFFECT. T. F. Yü, Peter G. Dayton*, Alexander B. Gutman* and J. J. Burns. Mt. Sinai and Goldwater Mem. Hosps., N.Y. and Natl. Heart Inst., Bethesda, Md.

Previous studies have demonstrated that analogues of phenylbutazone, more strongly acidic than the parent compound, have markedly enhanced uricosuric activity. By substituting methyl-sulfone groups in the para position of both benzene rings the pKa is reduced from 4.5 to 2.6, and the uricosuric action increased about 5-fold. However, the biologic half-life of this compound is concomitantly reduced from 72 hours for phenylbutazone to about 1 hour and the duration of uricosuric effect in acute renal clearance experiments is correspondingly transient. It has now been found that substitution of a methyl-sulfone group in the para position of only one benzene ring yields a compound with a pKa of 3.4, which possesses potent uricosuric properties, and has a biological half-life of 24 hours. This analogue in dosages of 200 mg two to four times a day reduces the high serum urate levels of gouty subjects to normal. The uricosuric effect persists for 2 or 3 days after discontinuance of the drug, in contrast to the rapid cessation of the uricosuric response to sulfinpyrazone (biologic half-life, 3 hours).

17 *Pharmacology*

TWO RAT DIURETIC TESTING METHODS. Virgil D. Wiebelhaus, Francis T. Brennan, Genevieve F. Sosnowski, and Andrew K. Polk (intr. by Arthur E. Heming). Res. & Dev. Div., Smith Kline & French Labs., Philadelphia, Pa.

We have evaluated two rat diuretic test methods; both methods are rapid and reliable. Using adult (200-400 g.) male albino rats (Wistar strain), Method I involves oral loading, via stomach tube, with distilled water (2.5 ml. per 100 grams of body weight). Test agents are suspended in the water load. Control groups receive only water. The rats are then placed in specially washed metabolism cages (4 rats per cage) and the subsequent five-hour urine output is collected, the volume measured and the sample analyzed for Na^+ content. Method II employs 0.9% NaCl as the 'loading' vehicle. Standard diuretic agents, 'Diamox', 'Diuril', 'Mercuhydrin Sodium', and 'Mictine' were tested by both methods. With Method I the Na^+ excretion values observed were found to be a sensitive and reliable index of the natriuretic activity of the 'standard diuretics'. In Method II both urinary Na^+ excretion and urinary volume were reliable indices of diuretic activity. Since urine volumes suffice, in Method II, as a reliable index of diuresis, this provides a rapid, simple test involving no special washing of cages and requiring no Na^+ analyses. The physiology and statistical aspects of both tests will be discussed.

18 *Pharmacology*

DIURETICS IN THYROIDECTOMIZED RATS. C. Miras (intr. by T. J. Haley.) Pharmacological Dept. Univ. Athens.

The diuretic effects of acetazoleamide, meralluride and mercaptomerin sodium were determined in intact and thyroidectomized rats. The completeness of thyroidectomy was checked by I 131 injection. Forty days after thyroidectomy, rats were given acetazoleamide by stomach tube, and the diuretic effect was measured against intact and thyroidectomized animals treated with urea. The experiment was also carried out by intraperitoneal injection of these drugs. The total amount of urine secreted in 24 hours was compared with that obtained with meralluride and mercaptomerin sodium. The diuretic effect of acetazoleamide was more pronounced.

231. RENAL FUNCTION–Electrolyte Excretion

1 *Physiology*

ROLES OF BLOOD AND URINE FLOWS IN DISTRIBUTING THO AND Na^{22} WITHIN KIDNEY. H. L. White, D. C. Tosteson and D. Rolf. * Dept. Physiol., Washington Univ. St. Louis.

Six minutes after clamping left ureter of a dog in osmotic diuresis, i.v. infusion of THO and Na^{22} is begun. At end of 5 minute infusion blood (femoral artery) and urine(right ureter) samples are obtained, renal pedicles clamped, and serial tissue samples from apex of pyramid to outer cortex of both kidneys are taken. In outer cortex specific activities (THO and Na^{22}) are the same in clamped (L) as in free (R) kidney and in arterial plasma, but in inner medulla L/R averages .30 for THO and .47 for Na^{22}. Urine is equilibrated with R inner medulla (THO and Na^{22}) and is 73% (THO) and 84% (Na^{22}) equilibrated with simultaneous arterial plasma. Better exclusion of THO than of Na^{22} from clamped medulla suggests better diffusion of THO across tops of vascular loops. Low THO values in early stop-flow samples previously reported must depend largely on poor medulla labelling via blood. Medulla on free flow side is labelled largely by downcoming urine. Cold Na in outer medulla of clamped side is 82% of free flow side. Apparently Na is washed out of clamped medulla by blood. (U. S. P. H. S. grant A-92)

2 *Physiology*

EFFECT OF A MERCURIAL DIURETIC ON SODIUM FLUX ACROSS PROXIMAL TUBULAR EPITHELIUM. A. Bisno, I. Kasser, D. Rolf, D. C. Tosteson and H. L. White (intr. by A. S. Gilson) Physiol. Dept., Washington Univ. St. Louis.

Sodium flux across the proximal tubular epithelium was estimated before and 1/2 hour after 3-4 mgm/kg of Hg as meralluride. During mannitol diuresis the left ureteral catheter was clamped; 6 minutes later constant infusion of Na^{22} into the left renal artery was begun. 8 or 9 minutes after clamping, the catheter was released and urine samples collected sequentially. Blood samples were obtained from the renal vein periodically during Na^{22} infusion and urine collections. New filtrate was signalled by creatinine. Measurements of plasma and urine Na^{22} specific activities made it possible to estimate Na flux between plasma and tubule. 9 animals showed a 1.5 to 3 fold rise in sodium flux after meralluride. In one case the effect was completely reversed by BAL. These results are consistent with an increased passive leak of sodium across the proximal wall after meralluride. This increased leak, rather than inhibition of pump processes, may be the mechanism by which mercurial diuretics increase urinary excretion of salt. (U. S. P. H. S. grant A-92)

3 *Physiology*

EXPERIMENTAL ASCITES WITH MARKED SODIUM RETENTION AFTER TRANSPLANT OF THE LEFT KIDNEY TO THE NECK AND RIGHT NEPHRECTOMY. Charles C.J. Carpenter*, John Holman*, Carlos R. Ayers* and James O. Davis (int. by Robert P. Akers), National Heart Institute, Bethesda, Md.

To evaluate the role of the renal nerves and of elevated renal venous pressure in the formation of ascites produced by thoracic inferior vena cava (IVC) constriction, experiments were performed on five dogs in which the left kidney and adrenal were transplanted to the neck, and the contralateral kidney was removed. Following these procedures, the thoracic IVC was constricted to the extent that IVC pressure increased 15 to 20 cm. of water. Subsequent to caval constriction, marked Na retention with rapid accumulation of ascitic fluid was observed in all animals; the low fecal Na/K ratio characteristic of dogs with experimental ascites with normally located kidneys and adrenals was noted, and urinary aldosterone excretion increased in the 2 animals in which measurements were made. Arterial pressure and plasma electrolytes remained within normal limits. In 1 dog, a natriuresis occurred when IVC pressure fell secondary to venous collateralization; in this animal, tightening of the caval ligature again resulted in virtually complete Na retention. These data indicate that neither renal nerves nor increased renal venous pressure are essential to the marked Na retention produced by thoracic IVC constriction.

4 *Physiology*

DIRECT RENAL ACTION OF CARDIAC GLYCOSIDES AND STRUCTURALLY RELATED COMPOUNDS. J.C. Strickler,* R.H. Kessler, and R.F. Pitts, Dept. of Physiology, Cornell Univ. Med. College, New York, N.Y.

This study amplifies previous observations that digitalis glycosides act directly on the kidney to increase water and electrolyte excretion. The mechanism of this action may be inferred from observations of Glynn and others who demonstrated that certain of these compounds inhibit ion movements across red cell membranes. In the present work some cardiac active glycosides and derivatives were infused into one renal artery of saline loaded dogs. Those compounds known to have cardiac toxicity or to affect ion fluxes increased Na^+, K^+, and water excretion. These effects could not be attributed to alterations in renal hemodynamics. At times Na^+ excretion exceeded 40% of the filtered load, indicating profound inhibition of sodium reabsorption in the proximal tubule. Several cardiac inactive compounds did not produce these changes despite a ten-fold increase in dosage. As also noted by Glynn, features common to the active compounds include an unsaturated 5 or 6 membered lactone ring and a specific steric relationship of the lactone ring to the C-17 carbon of the steroid nucleus. Our results demonstrate that only the cardiac active compounds increase urine flow and electrolyte excretion when infused into a renal artery.

5 *Physiology*

THE SECRETION OF POTASSIUM IN GOLDEN HAMSTERS. Klaus Hierholzer (intr. by R.C. Swan), Dept. of Physiology, Cornell Univ. Medical College, New York, N.Y.

Recent investigation of the site of secretion of potassium in dogs pointed to the same distal part of the nephron, where acidification and secretion of NH_3 occurs (Pitts et al. Am. J. Phys. 194:125, 1958). The latter activities were localized directly in collecting ducts of the golden hamster by Ullrich et al. (Pflügers Arch. 267:491, 1958 and 267:244, 1958) using the microcatheterization technique. However, they were unable to demonstrate any secretory activity for potassium at this site (Hilger et al. Pflügers Arch. 267:218, 1958). As part of an investigation of the site of K secretion in golden hamsters, simultaneous inulin and potassium clearance measurements have been performed. In female golden hamsters (110-130 gms) the inulin clearance has been measured at high inulin plasma concentrations, using Na sulfate as diuretic. Values ranged from 6.23-1.41 cc/min/kgm B.W. (average 3.84 cc/min/kgm B.W.). In all experiments in which potassium salts and acetazoleamide were administered, the simultaneous potassium clearance exceeded the inulin clearance (C_K/C_{inulin} ranging from 1.11-1.62). Under normal conditions potassium secretion could not be demonstrated.

6 *Physiology*

THE EFFECT OF AMMONIUM SALTS ON ELECTROLYTE EXCRETION IN THE CHICKEN. Luis Brenes*, Melvin Kahn*, Douglas Davidson* and Jack Orloff. National Heart Institute, Bethesda, Md.

The injection of NH_4Cl into the renal portal circulation of the chicken results in a unilateral rise in ammonia excretion on the injected side. This is associated with a profound natriuresis, chloruresis and an increase in urine flow. Potassium excretion fell when examined and urine pH generally rose. The inhibition of ion transport is specifically related to the administration of the ammonium ion. It is independent of the anion infused, occurring with the ammonium salts of sulfate, nitrate, acetate and chloride; it is neither produced by the corresponding sodium salts, nor by HCl or acetic acids. Alanine, an ammonia precursor, stimulates ammonia excretion but does not interfere with chloride reabsorption. The inhibitory effect of the ammonium salts appears to be dependent on the penetration and subsequent accumulation of NH_3/NH_4^+ within the tubule cells, since it is potentiated by alkalinization of the extracellular fluid and urine, a situation in which assuming free diffusion of the uncharged base, ammonia will preferentially accumulate within the tubule cell.

7 *Physiology*

THE DETERMINATION OF OSMOLALITY IN FROZEN KIDNEY SLICES. G. A. Bray (intr. by R. W. Berliner) National Heart Institute, Bethesda, Md.

In 1951 Wirz, Hargitay and Kuhn found that the thawing temperature indicated a progressive rise of osmolality throughout the inner medulla with the most concentrated slices at the tip of the papilla. A modification of this method has been used to study kidney slices from dehydrated rats and from rats undergoing water diuresis. Rat kidneys were frozen in liquid nitrogen; 10 μ slices were prepared and observed under polarized light. The thawing temperature was measured with a thermocouple. Slices from the inner medulla of dehydrated rats were consistently more concentrated than cortex and the loops consistently melted at a lower temperature than the collecting ducts. Scattered throughout the cortex were a number of tubules (presumably distal) that were hypotonic to cortex. These hypotonic structures were observed to the border of the outer medulla, but no hypotonic loops were ever seen within the outer medulla. In the water diuresis experiments the collecting ducts and distal tubules were both hypotonic to cortex while the loops in the inner medulla were hypertonic to cortex. The data suggest that any sodium transport by the thin ascending limb does not maintain osmotic gradients of more than 100-150 mOsm. The hypertonicity of medullary loops and interstitium appears to persist in water diuresis.

8 *Physiology*

CONCENTRATING ABILITY AND KIDNEY STRUCTURE. R. O'Dell* and B. Schmidt-Nielsen (introduced by K. Schmidt-Nielsen.) Dept. of Zool., Duke Univ., Durham, N. C.

According to the countercurrent hypothesis, the ability to concentrate the urine must be closely related to the length of the countercurrent system in the kidney. In most mammals there are both long and short looped nephrons, but some species have exclusively one or the other type of nephrons. The function of long and short looped nephrons as countercurrent multiplier systems was compared in a) beavers with short looped nephrons only, b) the African rodent Psammomys obesus with long looped nephrons only, and c) rabbits with a mixture of both. Maximum urinary osmolarities were: Beaver, 600 mOs; Rabbit, 1500 mOs; and Psammomys, 6000 mOs. In all three species an increasing urea and Na concentration was found from the border of the cortex through the outer zone of the medulla. In both rabbit and Psammomys the increase in concentration continued through the inner zone of the medulla. Apparently both short and long looped nephrons act as multiplier systems in the outer zone of the medulla. That the inner zone is also a multiplier system is indicated by the continued increase in Na concentration through this zone, and the close relationship between the thickness of the inner zone and the urinary concentrating ability. (Supported by American Heart Association and NIH Grant A-1956.)

9 *Physiology*

FUNCTIONAL DISTRIBUTION OF SOLUTES IN THE RENAL TISSUE OF RODENTS. B. Schmidt-Nielsen and R. O'Dell*. Dept. of Zool., Duke Univ., Durham, N. C.

How urea is handled in the renal countercurrent system is still an open question. In rabbits and in the African rodent Psammomys obesus loaded with urea (or on high protein diet), the urea concentration in the urine was up to 1000 mM higher than in the tissue water of any zone of the papilla. However, the total osmolar concentration of the urine was equal to that of the papilla tissue water because the concentration of electrolytes was higher in the tissue water than in the urine. In animals loaded with NaCl, or in animals on low protein diet, the urea concentration in the urine equaled the urea concentration in the papilla tissue water. Regardless of the urinary composition with respect to urea, Na, and K, a steady increase in Na concentration was found throughout the inner zone of the medulla. One possible explanation for the difference in urea distribution between urine and papilla tissue water during different functional states could be that the permeability of the collecting ducts to urea can change. (Supported by the American Heart Association and NIH Grant A-1956.)

10 *Pharmacology*

REDUCTION OF RENAL EFFECT OF ACETAZOLAMIDE (A) IN THE EXTRACELLULAR ALKALOSIS OF K+DEPLETION. Thomas H. Maren, Oliver A. Sorsdahl*, and Alfred J. Dickhaus*. Dept. of Pharmacology and Therapeutics, Univ. of Florida Coll. of Med., Gainesville.

Decrease in renal effect of carbonic anhydrase inhibitors in metabolic acidosis has been ascribed variously to low filtered HCO_3^- load, or to net body acidosis. K^+depletion furnishes presumptive means of separating these factors; plasma HCO_3^- is increased in presence of intracellular acidosis and low urinary pH. Beagles were given K^+free, high Na^+diet, and 1 mg/kg/day DOCA for 3 weeks. In a typical case plasma conc. (meq/L) were then Na^+,147; K^+, 3.1; Cl^-;105; HCO_3^-,30; pH,7.38; Urine pH was 5.7. Single i.v. doses of A (10 mg/kg) were given to the same dogs before, during, and 4 weeks after K^+depletion. Following are excretion rates 2 hrs. after A at each stage, in the dog cited:

Stage of Low K^+	Plasma CO_2 mM/L.	Na^+	K^+	Cl^-	HCO_3^-	GFR ml/min.	Excr./Filt. HCO_3^-, %
		microequiv./min.					
Before	21.6	42	37	12	59	19	16
During	31.8	11	33	5	17	14	4
After	22.2	70	25	13	61	20	15

During K^+depletion, effect of A on HCO_3^- output drops despite high filt. HCO_3^- load; excr./filt. HCO_3^- is similar to that after A in metabolic or respiratory acidosis. Unifying hypothesis is that renal response to these diverse types of acidosis is increased H^+secretion, independent of carbonic anhydrase. Role of enzyme and effect of inhibitor is therefore diminished.

11 *Pharmacology*

ADDITIVE NATURE OF THE RENAL EFFECTS OF AMINOPHYLLINE AND HYDROCHLOROTHIAZIDE. BOHDAN R. NECHAY. (INTR. BY T. H. MAREN). DEPT. OF PHARMACOLOGY AND THERAPEUTICS, UNIV. OF FLORIDA, COLL. OF MEDICINE, GAINESVILLE.

THE RENAL ELECTROLYTE EXCRETORY PATTERN OF AMINOPHYLLINE (AM) IN THE DOG WAS FOUND TO BE INDISTINGUISHABLE FROM THAT OF HYDROCHLOROTHIAZIDE (HC). THIS PATTERN IS THAT OF Cl^- MATCHED BY VARYING PROPORTIONS OF Na+ AND K+ IN ACID URINE. NEITHER DRUG SHOWS CHANGE OF ACTIVITY FOLLOWING ESTABLISHMENT OF METABOLIC ACIDOSIS OR ALKALOSIS, WHICH IS IN SHARP CONTRAST TO BEHAVIOUR OF MERCURIALS OR CARBONIC ANHYDRASE INHIBITORS. NEVERTHELESS IT WAS FOUND THAT AM (27 MG/KG I.V.) HAS AN ADDITIVE EFFECT TO MAXIMALLY ACTIVE (ON Cl^-) DOSES OF HC (5 MG/KG.I.V.). SINCE IT HAS PREVIOUSLY BEEN SHOWN THAT ACTION ON Na+ AND Cl^- REABSORPTION BY CHLOROTHIAZIDE (AND PRESUMABLY HC) IS SEPARATE FROM THAT OF MERCURIALS, WE POSTULATE THAT AM CONSTITUTES A THIRD MEANS WHEREBY DRUGS INFLUENCE CHLORIDE REABSORPTIVE SYSTEMS IN RENAL TUBULES. IT APPEARS THAT AT LEAST 3 DIFFERENT (ENZYMATIC?) PROCESSES CONTRIBUTE TO THE OVERALL MECHANISMS OF Cl^- AND Na+ REABSORPTION. (FELLOW OF AMERICAN HEART ASSOCIATION).

17 *Pharmacology*

THE EFFECT OF 5-HYDROXYTRYPTAMINE (5-HT) ON RENAL HEMODYNAMICS, WATER AND ELECTROLYTE EXCRETION. J. Maxwell Little, Emily A. Angell*, Ward Huffman* and William Brooks*. Bowman Gray School of Medicine, Winston-Salem, N. C.

Five unanesthetized female dogs, 9.5-13.5 kg, were studied in the post-absorptive state. NaCl, 0.9%, was infused throughout at a rate of 0.5 ml/kg/min. Following 45 min equilibration there were: two 15 min (A), three 20 min (B) and three 15 min (C) periods. Control experiments and those with 5-HT infusion during period (B), at 3 or 1.5 mcg/kg/min as free base, were run on each animal. Control and 5-HT experiments were compared for each group of periods for: Rates of GFR, RPF, urine flow, Na, Cl, and K excretion; and for tubular loads of Na, Cl, and K. The following differences in variables were significant with $P<0.02$. For periods (A) no differences were significant. For periods (B), there were significant decreases only for Na, Cl and K excretion rates with 3 mcg 5-HT; while with 1.5 mcg 5-HT there were significant decreases in Na and Cl loads and excretion rates. For periods (C), with 3 mcg 5-HT, there were significant decreases in RPF, urine flow, and Na and Cl excretion rates; while with 1.5 mcg 5-HT there were no significant decreases. In 27 periods (B) in control experiments the correlation coefficient between Na load and excretion = +0.129. Conclusion: 5-HT infusion may significantly decrease electrolyte excretion without significant effects on urine flow, RPF or GFR. (Supported by Grant H-2820, NIH.)

13 *Pathology*

ULTRASTRUCTURAL CHANGES IN THE RENAL PAPILLARY TIP WITH K+ DEPLETION. F. Straus* and B. Sparro, Dept. of Pathology, U. of Chicago, Chicago, Illinois.

Striking morphological renal changes pathognomonic of potassium deficiency are readily produced in rats kept on a diet deficient in this element. While different sites of morphological change have been described, recent studies have emphasized the predominance of alterations in the medullary portions of the nephron especially in the collecting tubule near the papillary tip. This conspicuous droplet formation has been said to represent altered mitochondria by analogy to the droplet change found in the proximal tubule in certain stages of protein resorbtion. Electron microscopic examination with magnification up to 30,000 diameters reveals changes suggesting that these droplets are unique. In addition, they have a remarkable zonal distribution. While they are most conspicuous in the collecting tubule cells, they are prominent in the interstitial and endothelial cells of the papillary tip. Much of the previously described P.A.S. positive material in this area can be shown by electron microscopy to be droplets with structure similar to those found in the collecting tubule cytoplasm. The unusual distribution of droplet change in all cells of the papillary tip with K+ deficiency suggests an association with the osmotic gradient proposed by the advocates of the counter-current theory.

232. RENAL FUNCTION–Glomeruli

1 *Physiology*

PERFUSION OF THE DOG KIDNEY IN SITU. Kendrick Hare, Francis Patton*, James Donahoo* and Ruth S. Hare*. Univ. of Ala. Med. Center, Birmingham, Alabama.

A diaphragm pump of variable stroke volume was used to perfuse the kidney through a cannula inserted into the renal artery through a carotid or femoral artery. The input of the pump was connected to a cannula inserted into the abdominal aorta through a femoral artery. Clotting was prevented with heparin. Urine from each kidney was collected separately through ureteral catheters. This permitted a comparison of the simultaneous function of the kidney supplied by the dog's own circulation with that of the other kidney perfused by the pump. Glomerular filtration rate was measured by creatinine clearances; tubular function by the creatinine and, sometimes, osmotic U/P. Perfusion was continued for as long as 4.5 hrs. with filtration rates in the normal range and creatinine U/P ratios as high as 500. In many cases, when the condition of the dog deteriorated, function was better in the perfused than in the control kidney.

2 *Pathology*

EXPERIMENTAL ACUTE GLOMERULONEPHRITIS IN THE RAT; ELECTRON AND LIGHT MICROSCOPIC STUDIES. J. Churg, B. Grishman and W. Mautner (intr. by H. Popper). Dept. of Pathology and the Cell Research Lab., Mt. Sinai Hosp., New York, N.Y.

Masugi-type nephritis was produced in rats by means of a single injection of anti-kidney rabbit serum,and renal changes studied at intervals from 2 hours to 6 weeks. During the first 3-4 days there was marked swelling and vacuolation of the epithelial and the endothelial cells of the glomerulus,accompanied by fusion and disappearance of the epithelial foot processes, and by obstruction of the capillaries by the swollen endothelial cytoplasm and "hyaline" (fibrin) thrombi. During this phase the animals developed albuminuria,oliguria,rapidly increasing edema and sometimes fatal uremia. In the 2nd phase (4-14 days) albuminuria reached a maximum and began to decline. The edema either cleared following the onset of diuresis,or progressed till the death of the animal. The cellular changes tended to regress,the foot processes reappeared,but at the same time the capillary basement membrane showed progressive thickening and splitting with eventual fragmentation of the outer split layer reminiscent in appearance of the "membranous transformation" seen in human nephrotic syndrome. Presence of cells and "fibers" in the intercapillary space after the 2nd week indicated gradual transition to chronic glomerulonephritis. (Supported by PHS Grant A918 & Kidney Dis.Fdn. of No. New Jersey).

233. RENAL FUNCTION–Tubules

1 *Physiology*

EFFECT OF SECRETION-REABSORPTION ON TITRATION CURVES OF TUBULAR TRANSPORT. G. H. Mudge, I. M. Weiner, K. Garlid* and J. Romeo*. Johns Hopkins Univ. School of Medicine, Baltimore, Maryland.

Various reports have shown that certain organic acids undergo net secretion at low plasma levels and net reabsorption at high. This phenomenon has been re-examined with the possibility that it might be evidence for simultaneous and continuous secretion and reabsorption. As a first approximation, tubular transport (T) was calculated assuming a secretory T_m, complete extraction up to T_m, and the passive reabsorption of a constant fraction of the amount entering the tubular lumen by both filtration and secretion. Calculated T showed net secretion at low plasma levels, net reabsorption at high. Consideration of further physiological parameters precludes an exact mathematical formulation. With salicylate and salicylurate this phenomenon was observed in dogs at constant urine volume and pH. Secreted sulfonamides had titration curves intermediate between PAH and salicylurate.

2 *Physiology*

THE ACCUMULATION OF URIC ACID BY RABBIT KIDNEY SLICES IN VITRO M. M. Platts* and G. H. Mudge (intr. by Philip Bard) Johns Hopkins Univ. School of Medicine, Baltimore, Md.

Slices of rabbit kidney cortex were incubated at 25°C in a saline medium containing urate and 10^{-2}M acetate. After one hour the urate concentrations in slice and medium were each measured enzymatically. The average recovery of added urate was 97.6%; hence, endogenous uricase appears to be relatively inactive. In oxygen, maximum slice/medium (S/M) ratios occur at an initial medium concentration of 0.7×10^{-3}M; average S/M ratio was 2.25. Lower or higher initial concentrations of urate produced lower S/M ratios. Incubation with succinate, citrate or α-ketogluturate decreased urate S/M ratios. Studies with other substrates are in progress. With anaerobic incubation average S/M ratio was 0.51. A similar degree of inhibition results (without reduction in oxygen consumption)with 2,4-dinitrophenol, probenecid and salicylate. The similarity between these results and those with p-aminohippurate suggests that urate and PAH may be accumulated and secreted by transport mechanisms having certain features in common. Studies on other species are in progress.

3 *Physiology*

RUN-OUT OF LUMINAL DIODRAST-I^{131} FROM ISOLATED RENAL TUBULES OF GOLDFISH. Wm. B. Kinter and Allen L. Cline*. Harvard Medical School, Boston, Mass.

Employing methods comparable to Hong and Forster's (J. Cell. and Comp. Physiol. 51:241, 1958), run-out of Diodrast-I^{131} from lumina of isolated goldfish tubules has been examined for evidence of a specific reabsorptive mechanism. Run-out of previously accumulated Diodrast is accelerated by metabolic inhibitors known to block luminal accumulation (secretion) of Diodrast. When employed simultaneously with these inhibitors, substances known to compete with Diodrast for secretion have the following effects: 1) those sluggishly secreted slow run-out; 2) those rapidly secreted further accelerate run-out. It is concluded that, with no metabolic energy for secretion, run-out depends on both passive "leakage" and carrier-facilitated diffusion with obligatory coupling between outward and inward diffusion. Competitive substances either block the carrier system by binding carrier (effect 1.) or drive it by inward diffusion (effect 2.). This carrier system may be an integral part of a secretory-reabsorptive mechanism. (Supported by Army Contract DA-49-007-MD-624).

4 *Physiology*

RETROGRADE MOVEMENT OF SOME NON-ELECTROLYTES ACROSS THE RENAL TUBULAR EPITHELIUM. J.C. Peña* and R.L. Malvin. Dept. Physiol. Univ. of Michigan, Ann Arbor, Michigan.

The stop flow technique was used to investigate the retrograde diffusion of non-electrolytes from the peritubular blood to the tubular lumen. 12 minutes after ureteral occlusion C^{14} labeled compounds were injected intravenously along with creatinine and PAH. 2 minutes later the occlusion was released and serial urine samples collected. If the radioactive compounds leak backwards their appearance can occur at any point along the nephron. In this manner we are able to localize the site of entrance by comparing the stop flow patterns for PAH, sodium and creatinine with that for the isotope. The rate of diffusion is measured by comparing the concentrations of the C^{14} labeled substances in the urine samples with that in the blood. The experiments demonstrated that urea and glycerol diffuse backwards along the length of the entire nephron, appearing in the urine before the simultaneously injected PAH and creatinine. The rate of diffusion of urea is higher than that of glycerol. Neither glucose nor mannitol are able to penetrate the distal tubule in retrograde fashion but seem to be able to diffuse slowly into the proximal tubular urine.

5 *Physiology*

RESPIRATORY QUOTIENT (RQ) OF DOG KIDNEY IN VIVO. Julius J. Cohen. Univ. of Rochester School of Medicine and Dentistry, Rochester, New York.

During a study of renal utilization of α-keto acids in anesthetized dogs, simultaneous renal O_2 consumption and CO_2 production rates were determined. In a majority of observations net CO_2 production exceeded net O_2 utilization, the mean renal RQ being significantly above unity. The renal RQ was also determined during NaCl infusion, acute metabolic alkalosis and acidosis. The mean (±S.E.) RQ's found were: (a) NaCl infusion (n=17), 1.33±0.07, (b) α-keto acid infusion (n=17), 1.27±0.08, (c) acute metabolic alkalosis (sodium lactate) (n=34), 1.18±0.08, (d) acute acidosis (n=32), 1.14±0.11. Variation of renal RQ is large within each group. However, the high statistical significance with which the mean RQ is above unity during NaCl and keto acid infusions indicates that renal CO_2 production is greater than O_2 utilization. These observations are interpreted to indicate that an anoxic region of the kidney derives some of its energy requirements from anaerobic coupled oxidation-reduction systems which produce CO_2. (Supported by USPHS Grant A-3602.)

6 *Physiology*

THE EFFECT OF DAMAGE TO THE FIRST PART OF THE PROXIMAL TUBULE ON ELECTROLYTE EXCRETION. T. Frederick Nicholson. Dept. Path. Chem., Univ. of Toronto, Toronto, Canada.

The injection of a 3 mg./100 ml. solution of potassium dichromate into one renal artery with removal of the solution through a puncture in the renal vein produces unilateral damage affecting approximately the first third of the proximal tubule of the dog's kidney. The damaged kidney excretes larger amounts of sodium, potassium, phosphate and bicarbonate and less hydrogen ion than the contralateral normal kidney. It is postulated that the reabsorption of sodium and potassium in the first part of the proximal tubule is in part an active process involving exchange with hydrogen ions. It appears that, although no change in the hydrogen ion concentration takes place in the upper part of the proximal tubule, normal acidification of the urine cannot occur if secretion of hydrogen ions in this part of the tubule is interfered with. This effect is probably due, in part at least, to excessive amounts of buffer reaching the more distal parts of the tubule. (Supported by a grant from the National Research Council of Canada).

7 *Physiology*

RENAL EXCRETION OF L-TYROSINE DERIVATIVES. K. C. Huang, UNIVERSITY OF LOUISVILLE, School of Medicine, Louisville, Ky.

Previous work in this laboratory has shown that in dogs L-tyrosine was reabsorbed by the renal tubules, while 3,5-diiodotyrosine was secreted. This study is an attempt to find out whether other derivatives of L-tyrosine behave similarly to the 3,5-diiodotyrosine with reference to their renal excretion. The following derivatives: 3-amino, 3-iodo-, 3,5-dibromo- and 3,5-dinitro-L-tyrosine were studied in dogs. In steady state experiments it was found that the two mono substituted derivatives of tyrosine were reabsorbed by the renal tubules and the two di-substituted derivatives were secreted. Experiments with ureteral stop-flow technique showed that the secretion of 3,5-diiodotyrosine was located in the proximal tubule and was blocked by administration of probenecid. It may be that the substitution of both hydrogen atoms in the 3,5 position on L-tyrosine, which will prevent the co-planarity of the phenyl and hydroxy groups, will induce tubular secretion of that compound.

(Aided by a Grant from the U.S.P.H.S. (A-2217)).

8 *Physiology*

GLUTAMINE AND KREBS CYCLE IN REGULATION OF RENAL AMMONIA PRODUCTION. William D. Lotspeich. Univ. Rochester Sch. of Med. & Dentistry, Rochester, N.Y.

The capacity to excrete ammonia is known to increase during acidosis in a number of species. It seemed apparent that factors besides a change in renal glutaminase activity are involved in this change. Therefore, a study was made of effects of pyruvate, α-ketoglutarate, malonate, and fumarate on free ammonia production from glutamine in homogenates of guinea pig kidney cortex. Pyruvate and α-ketoglutarate (30 μMol) markedly reduced free ammonia production. With pyruvate (but not with α-ketoglutarate) the addition of malonate (12 μMol) restored ammonia production to control levels. Further addition of fumarate (1 to 10 μMol) then resulted in a stepwise decrease in free ammonia. These results indicate that rate of amination of keto acids from glutamine may depend on differing levels of intracellular keto acids in alkalosis and acidosis. Thus keto acid level in tubular cells may play a role in adjusting the amount of intracellular ammonia available for diffusion into the urine. (Supported by a grant from the American Heart Association.)

9 *Physiology*

ELECTRICAL POTENTIAL MEASUREMENTS ON SINGLE NEPHRONS OF THE PERFUSED NECTURUS KIDNEY. Gerhard Giebisch, Dept. of Physiology, Cornell University Med. College, New York, N.Y.

Previous studies have demonstrated an average electrical transtubular potential difference of 20 mV, the tubular lumen being negative, and the existence of a potential difference of about 70 mV across the peritubular cell membrane of single proximal tubule cells. The doubly-perfused kidney of Necturus was found useful to test the effects of changes in the ionic environment on these potential differences. From observations on the degree of depolarization of single kidney cells at various concentrations of potassium in the luminal and peritubular fluid, considerable permeability of both the peritubular and luminal cell membrane of single proximal tubule cells to potassium is indicated. Substitution of sodium by choline or of sodium chloride by sucrose leads to significant reduction of the transtubular potential difference. From these findings and the fact that the transmembrane potential at the luminal cell side is 20 mV less than at the peritubular side, a higher permeability of the luminal cell membrane to sodium and active potassium uptake are believed to account for the greater reduction of a potassium diffusion potential at this site.

10 *Physiology*

MICROPUNCTURE STUDY OF RENAL TUBULAR ACIDIFICATION IN THE RAT. Erich E. Windhager and Gerhard Giebisch (intr. by John MacLeod), Dept. of Physiology, Cornell Univ. Med. College, New York, N.Y.

pH measurements were done on samples of proximal and distal tubular fluid using a quinhydrone electrode. The pH of arterial blood was estimated by means of a glass electrode. Strongest proximal acidification (0.8pH units) was found in animals kept on a low sodium diet and infused with isotonic sodium sulfate solution. A much smaller pH-drop of proximal tubular fluid was seen in animals under similar experimental conditions but kept on a normal diet. These findings are consistent with increased proximal hydrogen for sodium exchange under conditions favoring sodium conservation. Respiratory acidosis induced by 9% CO_2 resulted in significant proximal acidification. pH values lower than those of arterial blood were also found during osmotic diuresis induced by 20% mannitol. A smaller degree of proximal acidification was seen in animals infused with isotonic sodium chloride, and in animals kept on a low potassium diet. Since in the latter group pH values were not much different in proximal and distal samples, the collecting duct emerges as the main site of acidification in hypokalemic alkalosis. The results presented indicate a regulatory function of the proximal tubule in the process of hydrogen ion excretion and bicarbonate reabsorption.

11 *Physiology*

FUNCTIONAL DISCRIMINATION BETWEEN THE STRAIGHT AND CONVOLUTED SEGMENTS OF THE RENAL PROXIMAL TUBULE. J. B. Longley, M. B. Burg and H. J. Burtner (intr. by S. S. Spicer) Natl. Insts. of Health, Bethesda, Md.

Intravenous administration to rats of phenol red (PSP) or chlorphenol red (CPR) results in accumulation of dye in the outer stripe of the outer zone of the renal medulla. In kidneys of rats injected with dye during stop-flow, in kidneys perfused with dye solutions at pressures below the filtration threshold, or in kidney slices incubated in dye solutions, PSP and CPR accumulate in the renal cortex. These mutually exclusive distributions correspond respectively to the loci of the straight and convoluted segments of the proximal tubules, and represent accumulation of dye within these segments. The observations are taken to reflect excretion of dye by the convoluted segment and its reabsorption by the straight segment, tubular urine flow determining which pattern appears. This interpretation suggests that tubular reabsorption may account for extraction ratios less than 1.0 always observed for PSP and similarly handled compounds. The possibility of straight segment lesions enhancing net excretion in the PSP test is also indicated. Most significantly, the results unequivocally demonstrate the functional heterogeneity of the proximal tubule, a finding foreshadowed by the morphological heterogeneity long recognized in many vertebrates, including man.

12 *Physiology*

LOCALIZATION OF INTRARENAL OXYGEN TENSIONS BY STOP-FLOW. Donald W. Rennie. Univ. Buffalo Sch. Med., Buffalo, N.Y.

Urine oxygen tension (Pu) is considerably lower than that of renal venous blood (Pv). To localize the intrarenal site of urinary O_2 equilibration, e.g., cortex or medulla, the stop-flow technique has been applied in anesthetized dogs breathing 100% O_2. Pu was measured continuously as released urine flowed past a calibrated, bare-wire Pt electrode and was related to site of urine storage as determined by Cl^- and Diodrast I-131 concentrations. Pv was measured with a polyethylene-covered Pt electrode. Intrarenal-stored urine released at flow rates of 0.1 to 2 ml/sec had an almost uniform oxygen tension, averaging 100 mm Hg less than Pv measured during stop-flow. Pu of proximal tubular urine ranged from 65 to 160 mm Hg in 10 kidneys and averaged only 10 mm Hg more than Pu of distal urine. Pu of free-flowing urine corresponded closely with that of urine stored in proximal tubules. It is concluded that the low Pu of free-flowing urine results largely from equilibration with cortical tissue rather than with a small mass of inner medullary tissue. The results support the theory that tubules are perfused with blood low in red cell concentration. (Supported by a grant from the Life Insurance Medical Research Fund.)

13 *Physiology*

RENAL EXCRETION OF INORGANIC SULFATE IN THE CHICKEN. D. L. Globus*, E. L. Becker and D. D. Thompson. Dept. of Medicine, Cornell Univ. Med. College, New York City.

Previous work in dogs demonstrated that transtubular flux of radiosulfate from tubular capillaries to tubular lumen occurs. Because of the glomerular contribution to the excreted radiosulfate in the dog it was not possible to evaluate the effect of sulfate loading on the magnitude of this transtubular flux. By injecting radiosulfate into the renal portal circulation of one kidney in the chicken and collecting samples rapidly from each ureter, it is possible to examine the tubular flux on the injected side. Inulin, injected with radiosulfate, appears simultaneously and in similar quantities on both sides, but is preceded by radiosulfate on the injected side indicating that radiosulfate enters the tubule from the renal portal circulation. This obtains both before and after sulfate loading. The total radiosulfate excretion on the injected side exceeds that on the control side. In clearance experiments plasma sulfate was varied by infusion of sodium sulfate into a leg vein. The clearance of sulfate increases with increasing plasma sulfate. Though the peritubular plasma sulfate concentration is higher on the infused side, the clearance of sulfate is essentially the same in both kidneys at all systemic plasma sulfate levels studied (9-30 mM/l.).

14 *Physiology*

COMPOSITION OF LOOP OF HENLE AND COLLECTING DUCT FLUIDS IN THE HAMSTER PAPILLA. William E. Lassiter,* Carl W. Gottschalk and Margaret Mylle.* Dept. of Med., Univ. of N. C. School of Med., Chapel Hill, N. C.

Fluid was collected by micropuncture from loops of Henle, and collecting ducts at the exposed tip of the renal papilla in anesthetized, nondiuretic hamsters. The osmolality of each sample was determined, and also either its pH or the concentration of carbon-14 labeled urea or inulin carboxylic acid. The osmolality of loop fluid was essentially the same as that in adjacent collecting ducts. On the other hand, the pH of loop fluid was higher and nearly isohydric with plasma. The concentrations of labeled inulin and urea were much greater in the collecting ducts than in adjacent loops. These findings demonstrate that under these conditions, although their osmolality is the same, the chemical composition of loop of Henle and collecting duct fluids is different, consistent with the countercurrent hypothesis for urine concentration.

XUM

15 *Physiology*

EFFECT OF STROPHANTHIDIN ON TRANSPORT MECHANISMS OF THE RENAL TUBULE. J.R. Cade, R.J. Shalhoub, and M.L. Canessa (intr. by R.F. Pitts), Dept. of Physiology, Cornell Univ. Medical College, New York, N.Y.

That digitalis glycosides have a direct effect on ion transport has been shown by Glynn, Orloff, and others. This work amplifies the observation that strophanthidin has a direct effect on the renal tubule and studies in detail some of the transport systems involved. Strophanthidin in a dose range from 0.5 to 3.0 mgm was infused into one renal artery over an 80 min. period. A diuretic response which persisted following cessation of the infusion occurred in all experiments. The total Na excretion during and for 40 min. after the strophanthidin infusion was related to the dose of strophanthidin as was the per cent inhibition of Na reabsorption. A complete dose-response relationship could not be described due to depression of GFR which occurred when large doses of the drug were given. Studies of the effect of strophanthidin on K^+, Cl^-, titratable acid and other specific transport systems are in progress.

16 *Physiology*

EFFECTS OF CHELATING AGENTS ON ACTIVE TRANSPORT OF PAH BY RENAL CORTICAL SLICES. C. Larkin Flanagan* and David P. Earle, Jr., Northwestern University Med. School, Chicago, Ill.

Divalent cations are required by many enzyme systems and may be necessary for active transport of substances across cell membranes. The effects of chelating agents on active net accumulation of PAH by fresh rabbit renal cortical slices was utilized to assess the role of divalent cations in transport phenomena. A number of chelating agents affected PAH accumulation when added to the ambient medium at concentration of 1.8×10^{-3}M (pH = 7.6, 305 mosm./l, 24.5°C., 30 min). The most potent inhibitory chelators examined were DL-penicillamine and o-phenanthroline. These two compounds regularly depressed PAH slice to medium ratio by more than 50%. Oxygen consumption was not changed appreciably. The addition of chelates of penicillamine or o-phenanthroline (1 part metal: 1 part chelating agent) to the medium resulted in lessened inhibitory activity. In the case of penicillamine the inhibitory activity decreased gradually as the stability constant of the chelate increased. With o-phenanthroline, similar changes occurred, except that a very large decrease of inhibition occurred with the Zn^{++} as compared to the slightly weaker Cd^{++} chelate. Addition of metals alone indicated that Zn^{++} and Cd^{++} were not responsible for these changes. These results suggest that Zn plays an important role in active transport of PAH by kidney slices.

17 *Physiology*

FACTORS AFFECTING DIRECTION OF URINE-PLASMA GRADIENTS OF pCO_2 DURING PRODUCTION OF ACIDIC URINE. John T. Kaim * and William A. Brodsky. Univ. Louisville Sch. of Med., Louisville, Ky.

Unidirectional, maintained gradients of pCO_2 between urine and plasma have been found in dogs under conditions of respiratory acidosis and of elevation of serum phosphate to 3 mM/L. CO_2 tension of urine was 30-90 mm Hg. less than that of plasma, even in the presence of large amounts of carbonic anhydrase in urine. Stepwise elevation of serum phosphate to levels of 8 mM/L during respiratory acidosis resulted in obliteration of the pCO_2 gradient in 2 experiments. Elevation of serum phosphate (by infusion of NaH_2PO_4) to levels of 12-20 mM/L resulted in a reversal in direction of pCO_2 gradients, such that urine pCO_2 exceeded plasma pCO_2. On the basis of six experiments during formation of acid urine one could correlate direction and magnitude of pCO_2 gradients with level of serum phosphate. A relationship between urinary ammonia and pCO_2 gradients could not be established.

18 *Pharmacology*

RENAL TUBULAR SECRETION-REABSORPTION OF ORGANIC ACIDS. I. M. Weiner, John A. Washington II* and Gilbert H. Mudge. Johns Hopkins School of Medicine, Baltimore, Maryland.

Most previous attempts to establish structural requirements for the secretion of acids have been made without consideration of possible reabsorption. To evaluate certain parameters of renal secretion and reabsorption, the clearances of a number of organic acids were studied in dogs. The diversity of structure encountered among secreted acids (e.g. benzoates, hippurates, sulfonic acids, sulfonamides, cyclic amides, phenols, etc.) precludes any simple relationship between secretion and chemical configuration. Many acids ordinarily cleared at low rates undergo tubular secretion when the urine is alkaline. This secretion can be inhibited by PAH or probenecid. Within a given series of analogues, if pKa is constant, clearance varies inversely with the chloroform:water partition coefficients. However, if lipid solubility is constant, the clearance varies inversely with the pKa. Reabsorption, therefore, occurs by passive non-ionic diffusion. The data are interpreted as showing that many organic acids are actively secreted into the tubular lumen, but that the net clearance is determined both by secretion and by passive reabsorption.

19 *Pathology*

NATURE OF THE CELLULAR CHANGES IN RENAL COLLECTING TUBULES OF POTASSIUM DEFICIENT DOGS, MICE, AND RATS. Ashton B. Morrison, (Intr. by I.Zeidman) School of Med., Univ. of Pa., Phila., Pa.

Eosinophilic PAS positive granules have been reported in the renal collecting tubule cells of potassium deficient rats. In the present study similar changes have been seen in potassium deficient mice and dogs. The changes in the mice were more pronounced than those in the rats; those in the dog were much less clearly defined. On electron microscopic examination the granules in the collecting tubule cells did not appear to be altered mitochondria. Other cytoplasmic changes in the cells suggested that disordered osmotic relationships might be involved, and to test this, renal papillae from normal rats and mice were immersed in solutions ranging from plain water to 0.5 M sodium chloride. The changes produced in the collecting tubule cells were not those seen in potassium deficiency. There remains the possibility that the granules represent the aggregation of substances, perhaps of a mucoprotein nature, reabsorbed from the tubules. Evidence for this view is the fact that the mouse, which has ordinarily the greatest proteinuria of the three animals studied, had the most profound degree of granularity, while the dog, which has the least proteinuria, had few granules in its collecting tubule cells during potassium deficiency. (Supported by USPHS grants.)

234. REPRODUCTION

1 *Physiology*

EXHAUSTIVE ELECTROEJACULATION IN THE BULL. M. L. Hopwood* and F. X. Gassner. Endocrine Section, Colorado State University, Fort Collins, Colorado

To obtain information on the mechanism of ejaculation and on the nature of glandular fluids produced, 5 bulls (4 intact and 1 vasoligated) were subjected to exhaustive electroejaculation. Stimulation was applied at 3 min. intervals during periods ranging from 1-2 hrs. Seminal fluids (80-200 ml.) were collected in 20-36 fractions. Samples were cold-shocked and pH as well as levels of sperm, fructose, free amino acids, and nitrogen were determined. In fluids of intact bulls pH was found to be elevated in fractions showing low concentrations of these components; conversely, pH dropped whenever these levels increased. Sperm and glutamic acid were absent from fluids of the vasoligated bull; however, alanine, fructose, nitrogen, and pH were correlated as in the intact animal. Ejaculates collected by electroejaculation differed from those obtained by natural means (artificial vagina) in that volume and pH were increased, levels of the other components were decreased, and the protein electrophoretic patterns were altered, mainly due to loss of alpha-globulin components. Exhaustive electroejaculation had no adverse effects on subsequent semen quality or breeding behavior. (Supported by grants from USPHS (RG-3721) and USDA, ARS, Dairy Husbandry Research Service.)

2 *Physiology*

MAINTENANCE OF GESTATION IN THE RAT.[1] J. Yochim[2]* and M. X. Zarrow. Dept. Biol. Sci., Purdue Univ., Lafayette, Indiana.

Rats castrated on day 12 of pregnancy were injected with divided daily doses of the ovarian hormones and killed on day 20. Fetal survival (no. live fetuses/no. implant points) is found to be 4%, 49%, 74%, and 46% in rats treated with 1.0 mg, 1.5 mg, 2.0 mg, and 2.5 mg progesterone, respectively. Concomitant daily treatment with 0.1 μg estradiol increases fetal survival to 69%, 90%, 90%, and 69% in rats treated with 1.0 mg, 1.5 mg, 2.0 mg, and 3.0 mg progesterone, respectively. Injection of 0.2 μg estradiol further increases fetal survival to 61%, 97%, and 87% in rats treated with 0.5 mg, 1.0 mg, and 3.0 mg progesterone, respectively. A fetal survival of 96% and fetal weight of 2.26 g is obtained in the normal control rats. The greatest increase in fetal weight (2.86 g) is noted in rats treated with 0.1 μg estradiol and 2.0 mg progesterone. The effect of relaxin on the maintenance of gestation will be discussed.

[1]Aided by a grant from Ortho Res. Fdn. (PRF 1022).

[2]USPH Fellow, National Cancer Institute (CF 8558).

3 *Physiology*

IN VITRO OVARIAN AND ADRENAL METABOLISM OF GRAVID AND NON-GRAVID RABBITS AND GUINEA PIGS. J.F. Roux and S.L. Romney (intr. by A. Gilman) Ob.-Gyn. Dept., Albert Einstein Coll. of Med., Yeshiva U., N.Y. 61

Cl and C6 labeled glucose C^{14} was incubated with rabbit adrenal and ovarian slices by the method of Villee et al., Ped. 22, 954, 1958. During pregnancy, ovarian oxygen consumption doubled and respiratory quotient increased. Ovarian conversion of glucose 1 or 6-C^{14} to respiratory $C^{14}O_2$ increased four and twenty fold, respectively. Incorporation of both glucose to total lipids increased tenfold during pregnancy. Adrenal metabolic activity during pregnancy did not differ significantly from the non-pregnant state. Cytochrome oxidase and succinic oxidase activities in homogenates were determined manometrically. The activity of the two enzyme systems increased twofold during pregnancy in the ovaries of rabbits, but was unaffected in guinea pigs. It is concluded that ovarian, and not adrenal metabolic activity increases during pregnancy in species where ovarian dependency maintains gestation. (Supported in part by the Institutional Research Grant, Am. Can. Soc. and Grad. Train. Prog. (HTS-5408), USPHS.

4 *Pharmacology*

DEVELOPMENT OF SEMEN PRODUCTION IN THE GUINEA PIG. Matthew Freund (intr. by David Lehr). Dept. Physiology and Pharmacology, N. Y. Med. College, Flower-Fifth Ave. Hosps., New York City.

Methods for collection of guinea pig semen by electroejaculation and for its liquefaction by chymotrypsin were developed (Proc. Soc. Exptl. Biol. Med. 98:538) and used to examine semen from growing guinea pigs. Semen was collected by weekly electroejaculation, during weeks 4 - 20, from 23 guinea pigs. Mean ejaculate volume increased from 0.06 ml at 4 weeks to 0.76 ml, the mature level, at 18 - 20 weeks. Sperm appeared in the ejaculates of 2 pigs at 6 weeks, of half the pigs at 11 weeks, and of all pigs at 18 weeks. At 18 - 19 weeks, mean sperm concentration was 9,762,000 per ml, the mature level. Sperm motility was first observed at 11 weeks in the ejaculate of 1 pig and at 16 - 18 weeks in all ejaculates (10 - 80% motile cells). Mean body weight, at first appearance of sperm, was 611 gms; body weight was more closely correlated with sperm appearance than was age. Serial sacrifice of animals and histological examination of testes and accessory organs was made; correlations between histological examination and semen characteristics will be presented. (Supported by PHS RG-6099.)

5 *Pharmacology*

SPERMATOGENESIS IN FURAIROXYL TREATED RATS. George L. Ellman,* Robert M. Featherstone, V. Andres.* Dept. Pharmacology, U. Calif., San Francisco, Calif.

The effect of Furadroxyl(R) on spermatogenesis in the rat is cessation of sperm formation at the spermatocyte stage. The hypothesis that Furadroxyl may be a sulfhydryl reagent led us to try antagonizing its effects by simultaneous feeding of large amounts of cysteine. The rats were fed 0.15% Furadroxyl in the diet. The testes were removed, fixed in Bouin solution and stained with haematoxylin-eosin. Examination of these tissues showed that the drug treated tissues were uniformly damaged as described, whereas 1% cysteine in the diet prevented these pathological changes. Testicular tissue of normal rats was incubated (37°, anaerobic, glucose-Ringers, 2 hours) with and without Furadroxyl. The tissue sulfhydryl levels were determined. The decrease in the control tissues averaged 2.1 uM/g tissue. Those incubated with Furadroxyl decreased on the average 15.7 uM/g tissue. This decrease of -SH is 1.2 times the average Furadroxyl uptake of these tissues.

6 *Nutrition*

IRON CONTENT OF PLACENTAL TISSUE. Bonnie Amerman McCoy and Roberta E. Bleiler (intr. by Margaret A. Ohlson). Nutrition Dept., State Univ. Iowa.

Fifty intact placentae plus cord were obtained at random from the delivery room of University Hospitals. Iron was determined colorimetrically with α, α' dipyridyl in ashed samples. Sample weights ranged from 385 to 880 g. and the total iron content from 34.5 to 169.7 mg., with a mean of 75.5 mg. Per 100 g. of wet tissue, iron averaged 13.6 mg. with a range of 7.1 to 34.8 mg. One value, 89.0 mg. percent, was excluded since it was more than 6 standard deviations above the group mean; the mother received intramuscular iron prior to delivery. However, 5 other patients were treated with intramuscular iron, and several received ferrous sulfate tablets throughout pregnancy. In 5 instances, maternal hemoglobin before delivery was less than 10.0 g. percent; hemoglobin values did not appear to reflect placental iron content. The minimum iron loss due to expression of the placental material accounted for 3.5 percent of the suggested 0.9 g. requirement for additional iron during pregnancy while the maximum, exclusive of one of the 50 values as forementioned, was 18 percent of this need.

235. RESPIRATORY TRACT

1 *Physiology*

NON-ESTERIFIED FATTY ACID (NEFA) PRODUCTION BY RABBIT LUNG TISSUE IN VITRO. H.O.Heinemann, Dept. of Medicine, Francis Delafield Hospital, and Columbia University College of Physicians and Surgeons, New York City, N.Y.

It is known that mammalian lung tissue has lipolytic activity. To assess the nature of this process and establish its physiological significance, rabbit lungs were either perfused in vitro, or minced and incubated with suitable substrates containing albumen and coconut oil emulsion (Ediol). NEFA production was established by measuring the change in NEFA concentration. NEFA was determined by the method of Dole. The results indicate: 1. that NEFA production by minced rabbit lung tissue per gm. net weight is comparable to e.g., adipose tissue; and 2. that perfusion of the whole lung is associated with continuous rise in NEFA content of the perfusate. It is postulated that the lung may have functions other than to facilitate gas exchange and may play a role in fat metabolism of the body.

XUM

2 *Physiology*

PULMONARY PHYSIOLOGY AND MUCUS RHEOLOGY IN ADULTS WITH CYSTIC FIBROSIS. George Polgar and Robert Denton. (intr. by Robert D. Dripps). Dept. Physiol., Grad. Sch. Med. and Dept. Pediatrics, Sch. Med., Univ. of Pa., Phila., Pa.

It is generally believed that cystic fibrosis produces respiratory symptoms by obstructing the airways with an abnormal secretion. The disease is rarely found in adults. Four patients over 20 years of age were studied. One was a woman in the 6th month of her pregnancy. Two patients later came to autopsy. Volumetry of the lungs showed reduction of the vital capacity below 40% of normal; increase of the residual volume up to 580% of normal; increase of the total lung capacity in three cases. The resting ventilation was increased, the maximal breathing capacity less than 33% of normal. The maximal flow rates were reduced to 1/20 of normal. The distribution of ventilation was extremely uneven. The lung compliance was 1/2 - 1/10 of normal, the airway resistance up to 6-7 times the predicted value. The diffusion capacity of the lung was within normal range. In the arterial blood a moderate oxygen unsaturation, and an increased venous admixture with some CO_2-retention was found. In the viscometer the mucus had a very high resistance to initial flow and a high viscosity even after shearing. The pulmonary function was more deranged in these patients than has been reported by other workers.

3 *Pharmacology*

THE EVALUATION OF POTENTIAL ANTIASTHMATIC COMPOUNDS. M. Koukides*, S.A. Ferguson*, L.C. Greene*, and H. Wendel. Smith Kline & French Laboratories, Philadelphia, Pennsylvania.

Allergic asthma in guinea pigs resembles the disease in man much more than bronchiolar constriction induced by histamine, or by any other smooth muscle stimulant. Experimentally induced asthma in the guinea pig by an antigen-antibody reaction behaves similarly to human asthma in its response or lack of response to drugs in terms of respiratory changes. The following compounds were administered in varying concentrations as aerosols. The percentage of protection afforded by the compounds alone or in combination was calculated. 2-methyl-amino-1-hydroxy-1-(m-hydroxyphene)-ethane HCl (1% solution) afforded 44% protection, while SKF 1125-C (6,7-dihydroxy-1-isopropyl-1, 2,3,4-tetrahydroisoquinoline hydrobromide) afforded about 42% protection. By combining trimeprazine (rac-10-[3-dimethylamino-2-methylpropyl]-phenothiazine tartrate) with SKF 1125-C, a 56% protection is obtained. When the combination of SKF 1125-C and trimeprazine is evaluated at ½% solution instead of the 1%, protection is increased to 76%. Clinical experience has shown that, probably because of the complexity of the pathological mechanisms leading to the clinical symptoms, no single bronchodilator is satisfactorily effective in all cases. For this reason we have evaluated the potential of combination therapy, whose bronchiolytic activity is based on different mechanisms.

236. RESPIRATORY TRACT—Respiration

1 *Physiology*

NH_3 AS A NORMAL RESPIRATORY GAS. Eugene D. Robin, Philip A. Bromberg* and Claude E. Forkner, Jr.* Univ. of Pittsburgh Med. School, Pittsburgh, Pa.

The question of existence of NH_4^+-NH_3 *in vivo* in mammalian blood has been controversial. Dog lung has been used as an *in vivo* tonometer to resolve this controversy. The alveolar air of dogs receiving intravenous infusions of ammonium acetate contains measurable concentrations of free NH_3 gas ($PANH_3$ = 3 x 10^{-4} mm. of Hg). The pK'a of the NH_4^+-NH_3 system in the infused dogs averaged 9.13± 0.15. The alveolar air of dogs, not given ammonium, contained measurable concentrations of NH_3 ($PANH_3$ = 5 x 10^{-5} mm. Hg) and the pK'a did not differ significantly from that in the infused dogs (pK'a = 9.15± .49). It is concluded that NH_3 is a normal constituent of alveolar air arising as a result of diffusion from pulmonary capillary blood. Its presence in alveolar air thus indicates that NH_4^+-NH_3 is present normally in mammalian blood. (Supported by a research grant (H-2243) from the National Heart Institute of The National Institute of Health.

2 *Physiology*

SOME LIMITATIONS ON THE STUDY OF UNEVEN PULMONARY VENTILATION BY PRESENT METHODS: A THEORETICAL ANALYSIS. R.E.Nye, Jr. (intr. by Henry A. Schroeder) Dept. of Physiology, Dartmouth Medical School, Hanover, N.H.

The conclusion usually drawn from washout studies of normal lungs is that rather less than half the lung receives two or three times its share of the tidal volume. The present study uses difference equations to inquire (a) under what conditions lesser degrees of unevenness might be detected, if they exist, and (b) what factors might obscure the presence of small poorly-ventilated spaces. Calculations have been made for open and closed circuit methods, It is concluded that: (1) detection of minor degrees of unevenness is limited by the ability of the sensing device to distinguish between concentrations of indicator gas very close to the final concentration; (2)this resolving power must be greater, in open circuit, for any given degree of unevenness, when the poorly ventilated space is small than when it is large. This relationship is much less marked in closed circuit, and becomes reversed as evenness increases; (3)if mixing between regions occurs in the common dead space, the apparent size of the poorly ventilated space is increased; (4)a similar effect is produced by Pendelluft; (5)the last two effects are most marked when ventilation is only mildly uneven, and become less significant as unevenness increases.

3 *Physiology*

FURTHER EVIDENCE FOR INDEPENDENT, ADDITIVE ACTION OF ARTERIAL PCO_2 AND $[H^+]$ AS VENTILATORY STIMULI. Robert G. Stagman,* Daniel T. Johnson,* Thomas A. Stockert,* and John F. Perkins, Jr. Univ. of Chicago, Chicago, Ill.

Domizi, Perkins and Byrne (J.Appl.Physiol. 14:557, 1959) showed that with respect to levels in arterial blood, fixed acid (HCl) and CO_2 both act as potent ventilatory stimuli. Evidence for interaction between these stimuli was sought in this study by relating $PACO_2$ to $\dot{V}E$ (ordinate) before and after I.V. HCl. After acid the curve is nearly parallel to the control curve, but is shifted to the left, with lower value of $PACO_2$ at the horizontal intercept. CO_2 is effective as a stimulus even when below 40 mm after acid. Since curves of this type are predicted by the Gray equation, of the form: $\dot{V}A = K_1[H^+] + K_2PCO_2 - K_3$, these data, and those obtained by Nielsen (Skand.Arch.Physiol. 1936) in men before and after NH4Cl, support that portion of the Gray Theory which views arterial $[H^+]$ and CO_2 as independent, additive stimuli. This differs from hypoxia, in which slope of the PCO_2 vs $\dot{V}E$ curve increases (Nielsen and Smith, Acta Physiol.Scand. 1951, Lloyd Jukes and Cunningham, Quart.J.Exptl.Physiol. 1958) without change in intercept, indicating interaction between hypoxia and PCO_2, and requiring a multiplicative equation. (Supported by Public Health Service Grant No. H-1894 (C10) and the Wallace C. and Clara A. Abbott Memorial Fund.)

4 *Physiology*

CIRCULATORY AND RESPIRATORY ADJUSTMENTS TO EXPERIMENTAL DIVING IN THE ALLIGATOR. H.T. Andersen (intr. by H.T. Hammel) Univ. of California, Scripp's Inst. of Oceanography, La Jolla, Calif.

Conspicuous physiological adjustments to experimental submersion have been observed in the natural divers among mammals and birds. In order to obtain further information on the phenomena associated with water immersion and to explore these characteristics in other diving vertebrates, experiments have been carried out on the American alligator, Alligator mississippiensis. The heart rate was found to slow down during submersion, in some cases to only 5% of the pre-diving rate. The pressure induced in the large arteries by ventricular systole diminished only gradually during diastole due to a marked vasoconstriction. Lactic acid formed in the muscle during diving did not appear in the blood until after emersion, suggesting that the blood flow through the muscles is greatly decreased while the animal dives. The alligator may recover easily from a submergence lasting until the oxygen stores of the body are almost completely depleted. These results indicate that the "diving-reflexes" occur in a variety of vertebrates belonging to different phylogenetic groups. (Supported by a research grant from ONR, Contract Nonr-2216(09), and by Grant No. G-7476, Nat. Science Found.)

5 *Physiology*

INTERACTION OF HYPOXIA AND HYPERCAPNIA AT THE CAROTID BODIES IN CHEMOREFLEX STIMULATION OF BREATHING. Edward S. Otey and Theodore Bernthal (intr. by F.W.Kinard). Department of Physiology, Medical College of South Carolina, Charleston.

Arterial blood from one anesthetized dog (donor) was continuously circulated through the vascularly isolated carotid body region of another (the recipient) whence it was returned to a vein of the donor. For each, breathing was continuously recorded by spirometer, and end-tidal pCO_2 and pO_2 were under control while being monitored by infra-red and paramagnetic analyzers respectively. Hypercapnia in combination with normal end-tidal pO_2 in the donor evinced only slight increase in breathing by the recipient. When, however, hypercapnia of identical degree was superimposed upon a pre-existing hypoxia of the donor, there occurred a marked increment in the hyperpnea of the recipient beyond that due to the carotid body hypoxemia alone. Thus, combined hypercapnia and hypoxia at the carotid bodies of the recipient produced a substantially greater increase of breathing (about 100% on the average, but sometimes as much as 350%) than would be predicted by merely adding the increase due to separately imposed hypoxia to the increase due to separately imposed hypercapnia. It is concluded that the carotid body chemoreceptors are the site of a positive interaction between these two agencies such that the stimulating action of one is enhanced by the coexistence there of the other.

6 *Physiology*

BREATHING IN BRIEF EXERCISE. F. N. Craig and E. G. Cummings*. U. S. Army Chemical Warfare Laboratories, Army Chemical Center, Md.

Two Government employees ran for 20 or 60 seconds while inhaling air, oxygen or 4 per cent carbon dioxide. Inspired respiratory minute volume was determined for each breath. Ventilation increased suddenly in the first breath, with minimal changes in end-expiratory carbon dioxide tension and respiratory exchange ratio, to a rate that remained constant for 20 seconds before increasing further. The rate of carbon dioxide output was uniform during the first 20 seconds. A 12 per cent grade did not increase ventilation nor oxygen uptake during runs of 20 seconds, but in the first minute of recovery, ventilation was 64 per cent greater than after level runs. Inhalation of oxygen inhibited ventilation by 24 per cent in the 20-second periods before and after the end of a 60-second run. Inhalation of carbon dioxide begun at rest produced increments in ventilation and end-expiratory carbon dioxide tension that varied little during running and recovery. In the 20-second runs ventilation varied with speed but appeared independent of ultimate metabolic cost.

7 *Physiology*

INFLUENCE OF GAS MIXTURES ON A GROUPED TYPE OF PERIODIC BREATHING. Robert T. Schopp. Physiology Dept., Univ. of Colo. School of Medicine, Denver.

A grouped type of periodic breathing can frequently be induced in dogs under Pentothal-Chloralose anesthesia by giving additional injections of Pentothal. This pattern is characterized by two (occasionally three or more) respiratory efforts occuring in rapid succession followed by a longer apneic period. This couplet pattern is similar to Cheyne-Stokes breathing in that it is a regular irregularity. Its form differs from Cheyne-Stokes breathing in that there are fewer breaths per group, the breaths are of about the same amplitude and the breaths are apparently not as labored as are those at the peak of the group in Cheyne-Stokes breathing. The couplet pattern is not similar to Biot's breathing which is an irregular irregularity. The couplet pattern is eliminated by breathing 9% oxygen. In response to 10% carbon dioxide (21% oxygen) the pattern usually is either eliminated or the number of breaths per group increases. The pattern frequently persists while breathing 50-100% oxygen although the apneic periods are prolonged. This latter observation suggests that the couplet pattern involves a different mechanism from theCheyne-Stokes breathing which can be eliminated by breathing high oxygen.

8 *Physiology*

THRESHOLD TACHYPNEIC DIFFUSE PULMONARY EMBOLISM AND PULMONARY VASCULAR OBSTRUCTION. Theodore Bernthal, Alan D. Horres* and J.T.Taylor,III*, Department of Physiology, Medical College of South Carolina, Charleston, S.C.

Using 75u glass spheres as pulmonary emboli, the minimal dose range for simultaneously eliciting decreased tidal air and increased frequency of breathing in anesthetized dogs was found to be 0.0035 to 0.0085 ml./kg. with a mean of 0.0060 ml./kg. The degree of obstruction of the pulmonary vascular bed caused by such doses was ascertained by comparing pre-embolic and post-embolic blood volume-flow rates in the left lower lung lobe in situ during autoperfusion with arterial blood at selected uniformly maintained mean pressures. On the average, a mean threshold tachypneagenic dose of emboli diminished perfusion flow by 10 percent, interpreted as obstruction of this proportion of the left lower lobe vascular bed, and pre-embolic flow rate could be restored by an increase of pulmonary artery pressure of about 1 cm. H_2O. The small quantity of emboli required to elicit changes in breathing characteristic of diffuse embolic tachypnea and the relatively slight hemodynamic changes apparently entailed cast doubt upon hypotheses that altered pulmonary hemodynamics are essential in initiating this respiratory reflex. (Supported by Grant H-1320 from the National Institutes of Health).

9 *Physiology*

EXPERIENCE WITH A NEW RESPIRATORY CENTER STIMULANT

Sami I. Said (intr. by John L. Patterson, Jr.) Medical College of Virginia, Richmond.

Preliminary studies with a new respiratory center stimulant, vanillic diethylamide (Vandid*), have been carried out in dogs and in human subjects with obstructive emphysema and with extreme obesity. In the experiments on man, intravenous injection of the drug in doses of 0.3-1 mg/kg body weight caused a marked hyperventilation with a drop in arterial P_{CO_2} of 5-11 mm. Hg. The onset of hyperventilation, ushered by a sharp inspiration, followed the rapid injection in an arm vein by a period of about 20 seconds. The hyperventilation was largely due to an increased tidal volume. In addition to the respiratory stimulation, intravenous administration of the drug was followed by a moderate pressor response and a distinct rousing effect. Transient dizziness and hot flushes were noted. Experiments in dogs designed to determine the site of action of vanillic diethylamide showed it to be mainly on the carotid (and possibly the aortic) chemoreceptors. However, an additional direct action on the central chemoreceptors has not been ruled out.

* U. S. Vitamin and Pharmaceutical Corporation

10 *Physiology*

JOINT MOTION AND MINUTE VENTILATION IN NORMAL AND EMPHYSEMATOUS SUBJECTS. Herman F. Froeb (intr. by A. Baird Hastings). Scripps Clinic and Research Foundation, La Jolla, California.

Previous work by others has suggested a diminished sensitivity of the respiratory center in severe emphysema to the pCO_2 stimulus. The present experiments were designed to test the response to a different form of stimulation. Seven emphysematous subjects with hypercapnia(E) were compared to 10 normals(N) under basal conditions. This was followed by passive flexion and extension of the legs(I), mechanically induced, then with passive movement plus muscle contraction induced by electric stimulators(II), and finally with active leg exercise(III). The minute and alveolar ventilation in subjects E and N increased by 40% with (I). The oxygen cost of this increase in subjects E was three fold that in subjects N. There was no change in the arterial pCO_2 in subjects E and a significant fall in subjects N. It would appear that this reflex form of respiratory stimulation is intact in the emphysematous subjects but the metabolic cost of such stimulation is prohibitive, due to the increased work of breathing. (Supported by USPHS Grant IH-3528)

XUM

11 **Physiology**

EFFECTS OF LOW-FREQUENCY HIGH-AMPLITUDE VERTICAL SINUSOIDAL VIBRATION ON RESPIRATION IN HUMAN SUBJECTS. Lee R. Duffner* and Lyle H. Hamilton. Dept. of Physiol., Marquette Univ. Sch. of Med. and Wood Vet. Adm. Hosp., Milwaukee, Wis.

Although man is exposed to low-frequency whole-body vibration whenever he travels, little is known of the physiological effects of this environmental factor. The effects of sinusoidal vibration on respiration were investigated with 10 normal male subjects. Vital capacity, minute ventilation, breathing frequency, and expired air composition were measured before, during, and after a 4 minute exposure to vibrations of 2 to 7 c.p.s. at accelerations of 0.15 and 0.35 g. Oxygen consumption increased during vibration and dropped toward control values during the recovery period. Minute ventilation and tidal volume increased while breathing frequency decreased immediately after vibration began, remained unchanged during the entire period of exposure, and returned to control values as soon as vibration ceased. Vital capacity was not significantly changed during the vibration or recovery periods.

14 **Physiology**

CONTINUOUS OXYGEN DETERMINATION IN GASES BY THE POLAROGRAPHIC METHOD. Egidio Fulchiero* and Stevens J. Martin. St. Francis Hospital, Hartford, Conn.

Present methods are unsatisfactory for accurate, continuous determination of O_2 in gaseous mixtures. This study summarizes experiments in developing an adequa te method by a modification of the Clark electrode. The modified apparatus consists of a cylindrical glass cell, 20 x 4 mm, tapered at the free end to 2 mm, containing a small central platinum cathode embedded in a glass capillary, a Ag-AgCl anode recessed 3 mm from the tip of the cell, a supporting electrolyte solution of 0.15 M NaCl. The tapered end of the cell is covered by a Teflon membrane 0.0005" thick. The distal end of the cell is exposed to the gas to be analyzed and the proximal is connected to a polarograph and Rubicon galvanometer. Studies "in vitro" and in inhalation experiments in dogs and man revealed:(1) linearity of response and a high degree of accuracy (1-2% error) in a range 0-730 mm Hg pO_2, (2) rapidity of response, less than 10 seconds in a similar range of pO_2,(3) freedom from mechanical disturbances,(4) absence of "aging" up to 5-6 hrs of continuous use,(5) slight sensitivity to temperature changes (1.5-3% per °C). The apparatus is being used in studies on respiration.

17 **Physiology**

THE TRANSIENT EFFECT OF SUDDEN MILD HYPOXIA ON RESPIRATION. Thomas F. Hornbein* and Albert Roos. Lab. of Thoracic Physiol., Washington Univ. School of Med., St. Louis, Mo.

Recent studies by us and others of carotid body chemoreceptor activity in cats show that the activity of these organs increases markedly at a range of alveolar pO_2 considerably higher than the alveolar pO_2 of 50 to 60 mm.Hg. known to produce an increase in ventilation. To attempt to explain this discrepancy between chemoreceptor activity and ventilation, the transient ventilatory response to two breaths of nitrogen was observed and correlated with the minimum alveolar pO_2 during this brief hypoxic stimulus. This transient increase in ventilation could be detected at an alveolar pO_2 considerably higher than the highest pO_2 required to stimulate breathing during the steady state. These results suggest that at relatively high alveolar oxygen tensions the effect of chemoreceptor activity on ventilation is inhibited by other factors which only fully appear after several breaths. (Supported by a grant from the United States Public Health Service, H-82(C10).

15 **Physiology**

THE EFFECTS OF FEVER ON THE PULMONARY DIFFUSING CAPACITY AND PULMONARY MECHANICS IN MAN. L. Cander and E. Hanowell (intr. by E. P. Radford, Jr.) Dept. of Med., Tufts Univ. School of Med. and the New England Center Hospital, Boston, Mass.

Fever was induced in normal adult males by the intravenous administration of typhoid vaccine. Prior to the vaccine injection, control measurements were made of the oral temperature, pulse rate, respiratory rate (f), minute ventilation ($\dot{V}_E$), O_2 consumption ($\dot{V}_{O2}$), CO_2 production ($\dot{V}_{CO2}$), single breath D_{CO}, and lung mechanics as measured by the esophageal balloon technique. The measurements were repeated during the chill phase, peak temperature, defervescence, and when the temperature had returned to or near the control value. Although the predicted increases occurred in f, $\dot{V}_E$, $\dot{V}_{O2}$, and $\dot{V}_{CO2}$ with fever, there were no significant changes in the D_{CO}, or lung compliance measured at varying frequencies, or total pulmonary resistance. These preliminary studies suggest that the D_{CO} and lung mechanical properties are not altered significantly by temperature increase or the increased pulmonary blood flow associated with fever. (Supported by a grant from the Mass. Heart Association.)

13 **Physiology**

CARBON MONOXIDE DIFFUSION CAPACITY IN SEVERE EMPHYSEMA. Hurley L. Motley and Roman Yanda*, Univ. South. Calif. School of Med. Los Angeles, Calif.

The CO diffusion capacity is decreased in emphysema. Factors related to the decrease have been studied. Lung volumes and pulmonary ventilation measurements were obtained on 76 cases (residual volume above 200% of predicted, residual % of total lung capacity above 50% and with significant impairment in spirograms and air distribution), along with the CO diffusion capacity (steady state method). Step-up exercise values were obtained in 58. The resting CO diffusion capacity varied from 3-14 and step-up exercise 5-33, and a considerable overlap was noted in the emphysema group as compared to subjects with normal lung volumes. No correlation was noted for the CO diffusion with minute ventilation at rest, but with exercise a slight correlation was present. On 34% and 17% O_2 breathing the CO diffusion was decreased as compared to air breathing, and with only slight decreases in minute ventilation on 34% O_2, but minute ventilation was increased on 17% O_2. Exercise oxygen uptake was best correlated with CO diffusion. (Supported in part from grant H-1604 (C5) U.S. Public Health Service).

16 **Physiology**

4TH VENTRICLE RESPIRATORY CHEMOSENSITIVITY AND THE AREA POSTREMA. R.A.Mitchell, W.Massion, C.T.Carman, and J.W. Severinghaus (intr. by L.L.Bennett). Cardiovascular Research Inst., U. of Cal. Med. Sch., San Francisco.

Loeschke et al have described a superficial pH sensitive area in the 4th ventricle of cats which can influence respiratory tidal volume. We perfused the 4th ventricle of chloralosed dogs with mock spinal fluid of known P_{CO2} and pH at 38°. We varied the HCO_3^- keeping the P_{CO2} constant and maintaining isotonicity by altering Cl^-. In 18 dogs, ventilation was increased 350% within 20 minutes after changing the perfusate from 45 mM/L to 15 mM/L HCO_3^-. Perfusate P_{CO2} was held at 51 mm Hg and alveolar P_{CO2} was held constant by regulating inspired CO_2. Varying perfusate P_{CO2} widely, while keeping HCO_3^- constant, produced no consistent effects. We confirmed Loeschke's observation that this response was virtually eliminated by unroofing the 4th ventricle in 5 dogs. Preliminary experiments were done to identify and localize chemosensitive areas. All respiratory response to perfusion was abolished after discrete cauterization in the region of the area postrema in 5 dogs. We are studying the role of this region in the hyperpnea of CO_2 breathing in relation to other chemoreceptors. (Supported by USPHS RG-5881 and H-3961)

17 **Physiology**

PULMONARY VENTILATION IN OXYGEN DEFICIT OF NEWBORN. E.F. Adolph and P.A. Hoy*. Univ. of Rochester, Rochester, N.Y.

Newborn mammals survive prolonged lack of oxygen. Does survival depend on total oxygen deficit? Can hypoxia drive the breathing? The unanesthetized infant rat in a body plethysmograph breathed through a tracheal cannula. Body temperature was maintained at 34°C. The metered effluent air was analysed for oxygen. When pO_2 of 40 mm. Hg was inspired, oxygen consumption was only one-fourth of that in air for at least 1 hour. Subsequently no oxygen debt appeared. The total oxygen missed (deficit) during survival time was 4-6 fold greater in pO_2 of 40 than in pO_2 of zero. Hence no fixed deficit of oxygen led to death. During hypoxia the pulmonary ventilation of the newborn accelerated for a few minutes only. Heart beats slowed. Subsequently, while air was breathed, oxygen consumption and pulse recovered slowly during an hour. Lack of compensation by increase of breathing implies that oxygen did not continue to drive the ventilation, nor did carbon dioxide. Even in air, ventilatory coefficients were far from constant. Indifference to oxygen and carbon dioxide suggests the existence of some other breathing control, possibly centrogenic. On the first two days after birth the breathing pattern was irregular or pneumotaxic, but hypoxia makes it regular.

18 **Physiology**

THE EFFECT OF A NEW ANTITUSSIVE ON THE TRACHEAL-CLAMPING REACTION. Paul S. Guth and Marvin Goldenberg (intr by J. C. Scott) Department of Pharmacology, Hahnemann Medical College, Phila. Pa.

When a cannula inserted in the trachea of a dog is clamped toward the end of inspiration, there is an immediate cessation of respiratory movement and a relatively slow increase in intrapleural pressure. Following this period of apnea, the intrapleural pressure falls and respiratory movements begin. At this point the tracheal clamp is removed. This reflex is presumed to be mediated via the pulmonary stretch receptors. It was therefore chosen as the test situation to determine whether a new antitussive (-phenyl-O-chlorophenyl 3-dimethyl aminopropanol -1 hydrochloride, SL-501) has as its site of action, the stretch receptors. Its effectiveness was compared with benzonatate. Both benzonatate and SL-501 were found to increase the duration of the apneic period. Discussion of the possible mode of action of SL-501 and similar drugs in the light of these findings will be presented. This was interpreted to indicate stimulation rather than inhibition as reported by Boucher (Schw. Med. Woch. 86, 94, 1956). The relationship of this effect to the other components of the cough reflex will be discussed.

237. RESPIRATORY TRACT—Respiration—Mechanics

1 **Physiology**

THE OXYGEN COST OF BREATHING. ESTIMATES BASED ON RESPIRATORY MUSCLE FORCE. Maurice McGregor and Margaret R. Becklake* Joint Cardio-Respiratory Unit, Royal Victoria Hosp. and Montreal Children's Hosp., Montreal, Canada.

There is a roughly linear relationship between oxygen cost of breathing and esophageal pressure in any one normal subject. There are different slopes relating the oxygen cost to esophageal pressure for hyperventilation on the one hand and for breathing against added resistance on the other. Correction for the component of pressure exerted against the chest wall reduces them to a single slope. When the pressure is multiplied by a rough estimate of lung surface area an index of force is obtained. This has roughly the same slope in normal subjects and patients with heart and lung disease. From this slope and the esophageal pressure one may obtain a rough estimate of the oxygen cost of breathing during physical exercise in normal subjects and in patients with heart and lung disease.

2 **Physiology**

DETECTION OF ADDED ELASTIC IMPEDANCE TO BREATHING. E.J.M. Campbell, S. Freedman, P.S. Smith, and M.E. Taylor. (Introduced by R.H. Shepard.) Medical Unit, Middlesex Hospital, London, England.

Six normal subjects were made to inspire unknowingly and in a randomised order from rigid drums of variable total volume and to signal if they detected the change. The mean 50% detection level was 2.47 cm. H_2O/l. (S.D. 0·408, n=12). There was no significant difference on repetition. These levels of impedance caused no significant or consistent change in tidal volume. Increasing the tidal volume by adding dead space enabled smaller impedances to be detected by most but not all subjects. It would therefore appear that these subjects could detect a 10-20% change in thoracic compliance. The mechanism by which detection occurred cannot have been stimulated solely by changes in tidal volume, transpulmonary pressure or transthoracic-cage pressure. The mechanism seems to require continuous correlation of pressure change and volume change, or of some related factors such as tension change and length change in the respiratory muscles. (Both forms of information could not have come from the lungs.) Whatever the mechanism the sensation seems to reach consciousness as a change of impedance.

3 **Physiology**

THE MECHANICS OF RESPIRATION DURING FORWARD ACCELERATION. J.F. Watson, Capt., USAF(MC), N.S. Cherniack, Capt., USAF(MC), and F.W. Zechman, Jr., (intr. by E.P. Hiatt, M.D.) Acceleration Section, Biophysics Branch, Aerospace Med. Lab., WADC, Wright-Patterson Air Force Base, Ohio.

The main factor limiting tolerance to acceleration where the inertial effect is transverse to the long axis of the body and directed front to back is difficulty in breathing. Therefore, the effect of forward acceleration on respiratory mechanics was studied. Static relaxation pressure volume curves of the thorax and lung were obtained on 4 trained subjects while in the supine position on the human centrifuge at 1, 2, 3, and 4g. As acceleration increased, the intrapulmonic pressure increased for a constant volume shifting the pressure volume curve downward and to the right. Compliance of the thorax and lung did not change from 1 to 4g, but elastic work of inspiration increased 280%. Inspiration during 4g acceleration, regardless of the end expiratory position, was always active and complete relaxation of the inspiratory muscles did not occur. Absolute lung volumes during acceleration decreased proportionately. Pressure volume relationships obtained during negative pressure breathing in a Drinker respirator and during forward acceleration were similar. It is suggested that forward acceleration physiologically resembles negative pressure breathing.

4 **Physiology**

RESPIRATORY CONSEQUENCES OF PASSIVE BODY MOVEMENT. P. B. Stewart,* M.E. Dixon,* D. V. Bates, and R. V. Christie. Joint Cardiorespiratory Service, Royal Victoria Hospital and Montreal Children's Hospital, Montreal, Quebec.

An "Exercycle" was modified to enable the following body movements to be studied independently. 1.Passive movement of legs on motor driven pedals. 2.Vertical trunk movement through 4", 30 or 48 times per minute. 3.Passive backward and forward movement of the body torso. 4.Vertical movement of the subject lying horizontally. 5.Various combinations of these movements. Measurements have been made of ventilation, oxygen uptake, and end tidal CO_2 concentration in a group of normal subjects. Observations during passive movement were preceded and followed by control periods at rest. Passive trunk movement vertically in a seated subject produces considerable overventilation with regard to CO_2 production; at a seat movement of 48 per minute, the ventilation is nearly doubled, and the CO_2 tension may drop as low as 22 mm.Hg. The respiratory rhythm does not seem to bear a constant relationship to the phase of the seat movement. These findings are believed to explain the hyperventilation experienced by jet pilots, previously ascribed to psychogenic factors (BALKE et al., J.Aviation Med., 28, 241, 1957). Pedal movement alone produces a balanced increase in ventilation and oxygen uptake with no change in end tidal CO_2 tension.

5 *Physiology*

PARTITION OF PULMONARY FLOW RESISTANCE INTO UPPER AND LOWER AIRWAY COMPONENTS. Robert E. Hyatt and Roger E. Wilcox*. Beckley Memorial Hospital, Beckley, West Virginia.

Separation of the resistance of the lower airways and lung from that of the upper (extrathoracic) airways has physiologic importance. 11 unanesthetized male subjects were studied. Lateral intratracheal pressure was measured by an 18 g. trochar introduced percutaneously 3 cms. below the vocal cords. Intrapleural pressure was estimated by an esophageal balloon. Lateral oral pressure was measured distal to the lips in a pipe of 19 mm. I.D. The pressure drops from esophagus to trachea and trachea to mouth were recorded simultaneously with flow and spirometer volume. All measurements in a given run were at the same degree of lung inflation. During inspiration the upper airway contributed 29.2% (ave.) of the pressure drop from esophagus to mouth and 19.4% during expiration. Flows up to 3.5 L./sec. were studied. A visual straight line fit of the data yielded an average upper airway resistance of 1.0 cms./L./sec. (inspiration) and 1.1 cms./L./sec. (expiration). Marked variability occurred in some subjects. Preliminary data indicates that the position of the needle in the airstream had little effect. Upper airway resistance represents a significant portion of total lung resistance in normal subjects. (Supported by grants from USPHS and MMHA.)

6 *Physiology*

VENTILATORY RESPONSE TO FORWARD ACCELERATION. Fred Zechman, Jr. Neil S. Cherniack, Capt., USAF(MC) and Alvin S. Hyde, Capt., USAF (MC) (intr. by W. C. McNelly.) Aerospace Medical Laboratory, Wright Air Development Center, Wright-Patterson Air Force Base, Ohio.

One of the primary factors limiting man's tolerance to forward acceleration is associated with the difficulties encountered in breathing. To further examine this problem, two series of experiments were conducted. In the first, the effect of 5, 8 and 12g on frequency, tidal volume, minute volume and nitrogen elimination was determined. In the second, oxygen consumptions were measured. In both series of studies, the trunk was inclined 12° in the direction of acceleration and a rate of onset of 1g per second was used. Frequency increased reaching an average of 39.2 cycles per minute and tidal volume decreased to an average of 318 cc at 12g. Nitrogen elimination at 12g did not change significantly suggesting that gross alveolar ventilation was unchanged. Oxygen consumptions increased with acceleration and it is presumed that the extra work of breathing is an important contributing factor.

7 *Physiology*

INSPIRATORY AND EXPIRATORY VITAL CAPACITY TIME (IVCT AND EVCT) OBTAINED FROM BREATH VELOCITY-VOLUME LOOPS. R. G. Bartlett, Jr., and N. E. Phillips* Physiology Department, U. S. Naval School of Aviation Medicine, Pensacola, Fla.

If breath velocity and breath volume signals from the Servo-Spirometer are fed into the vertical and horizontal axes, respectively, of a Cathode Ray Oscilloscope, a velocity-volume loop results. The area of this loop (A) = velocity ($\dot{V}$) x volume (V). If V/unit time (T) is substituted for $\dot{V}$ and the terms are rearranged, "Time" may be calculated from the equation $T = V^2/A$. Area may be either inspiratory or expiratory, and the formula then defines an inspiratory or expiratory "time." "Time" calculated by this formula is a physiologic rather than a chronologic elapsed time and is altered little by straining at the end of the inspiratory or expiratory effort. When airway resistance is artifically increased, V is unchanged, $\dot{V}$ is lowered, and T is increased. This Inspiratory or Expiratory Vital Capacity Time (IVCT or EVCT) may be used in pulmonary function evaluation and presents, among other things, information usually obtained from Timed Vital Capacity and Maximum Breathing Capacity Tests.

8 *Physiology*

THE EFFECT OF RESPIRATORY TRACT OBSTRUCTION UPON THE VENTILATORY RESPONSE TO INHALED CO_2. Philip Samet, Eugene Fiener*, and William Bernstein*. Mt. Sinai Hospital and Univ. of Miami School of Med., Miami Beach, Fla.

The factors responsible for the depressed ventilatory response to inhaled carbon dioxide in patients with emphysema and hypercarbia remain a subject for intensive study. The purpose of this report is to delineate the effect of respiratory tract obstruction upon the ventilatory response to inhaled carbon dioxide in normal subjects. Ventilatory and gas exchange data and arterial blood data were measured in 14 normal subjects before and during interposition of varied grades of respiratory tract obstruction during inhalation of compressed air and 3 and 5% CO_2 in compressed air. Maximum breathing capacity was also determined. The milder degree of respiratory obstruction resulted in no significant alteration in alveolar ventilation and arterial blood gas data despite a decrement in maximum breathing capacity from a control level of 150L./Minute to 44L./Min. The more severe degree of obstruction produced significant decrements in alveolar ventilation and increments in arterial blood carbon dioxide tension levels. The corresponding maximum breathing capacity fell to 32L./Min. These data suggest that respiratory tract obstruction does not decrease the respiratory response to inhaled CO_2 until the degree of obstruction is advanced— i.e., the decrease in CO_2 response is a "breaking point" phenomenon rather than one in which minor degrees of obstruction cause slight decrements in CO_2 response.

9 *Physiology*

STATIC PRESSURE-VOLUME CHARACTERISTICS OF LUNGS IN NORMAL MALES. Solbert Permutt and Harry Martin (intr. by John L. Chapin). Depts. of Medicine and Environmental Medicine, Johns Hopkins Univ., Baltimore, Md.

The static pressure-volume characteristics of the lungs were determined in 28 normal men between the ages of 21 and 76. Transpulmonary pressure (the pressure difference between the mouth and surface of the lungs) under static conditions was determined by recording the pressure difference between the mouth and an esophageal balloon when breathing was interrupted for approximately 0.1 seconds. Transpulmonary pressures were measured in relation to absolute lung volumes throughout the entire range of the vital capacity, both on inspiration and expiration. There was an increase in residual volume and a decrease in vital capacity, but no change in the slope or position of the pressure-volume curves with advancing age. The older subjects had higher transpulmonary pressures at residual volume and lower transpulmonary pressures at total lung capacity. The results suggest that with advancing age there is little change in the intrinsic static pressure-volume characteristics of the lungs themselves and that the increase in residual volume and the decrease in vital capacity with advancing age are more likely due to changes in either the compliance or muscle power of the thorax.

10 *Physiology*

FACTORS DETERMINING STEADY STATE CONTRACTIONS OF ABDOMINAL MUSCLES. W. B. Youmans, L. D. Davis*, J. K. Turner* and D. I. Briggs*. Department of Physiology, University of Wisconsin, Madison.

In anesthetized dogs the abdominal muscles show steady state contractions which are inhibited during inspiration and reappear at the termination of expiration (Gesell). The steady state contractions are increased by 1) a rise in pCO_2 of alveolar air, 2) hemorrhage, 3) injection of vasodilator agents, 4) positive pressure breathing; and they are decreased by 1) hyperventilation, 2) negative pressure breathing, 3) transfusion and 4) injection of vasoconstrictor agents. The activity of abdominal muscles, as determined by electromyography, can be made to disappear entirely by combining hyperventilation with procedures which increase the central blood volume. The abdominal compression reaction is a type of activity seen whenever the steady state contractions are of considerable amplitude. The amplitude of ACR is determined in part by factors which are not concerned with determining the amplitude of inspiration. On occasion during recovery from hyperventilation several rhythmic abdominal contractions may occur before respiration is resumed. Implications of this result will be discussed.

11 *Physiology*

EFFECT OF ACUTE CENTRAL VASCULAR CONGESTION ON PULMONARY COMPLIANCE OF NORMAL SUBJECTS. Stuart Bondurant*, Jere Mead, C.D. Cook*. Dept. Physiol., Harvard Sch. Pub. Health, Boston, Mass.

The decrease in pulmonary compliance which has been reported in human studies during central vascular congestion is greater than that produced in animals under similar conditions. Reportedly, extrinsic pressure on the esophagus may cause respiratory intraesophageal pressure change to be greater than intrathoracic pressure change. To evaluate the possibility that similar pressure effects during central vascular congestion may have contributed to the apparently low compliance observed in the human studies, central congestion was produced by inflation of a g-suit on seated normal subjects and the following observations were made: With the esophageal balloon in the distal esophagus and with no control of the reduction in FRC caused by g-suit inflation, compliance apparantly decreased by 50%; with the balloon in mid-esophagus, and with FRC voluntarily held constant, compliance decreased by 25%, intraesophageal pressure was never more negative at high lung volumes and was consistently more positive at low lung volumes. Conclusion: There is evidence of intermittent extrinsic esophageal pressure during central vascular congestion in seated subjects. When this is reduced, compliance changes induced by central vascular congestion are similar in magnitude in man and animals.

12 *Physiology*

STUDIES ON COLLATERAL VENTILATION. H. B. Martin and F. Holmstrom.* Dept. of Physiology, Harvard School of Public Health, Boston, Mass.

The first two branches of the bronchus of a single lobe of excised dog lungs were separately cannulated, and air from a spirometer under constant pressure was allowed to flow into one of these branches and out the other via the pathways of collateral ventilation. Flow and the pressure difference between the two cannulated bronchi were measured and flow resistance calculated. Pressure-flow curves were straight lines indicating laminar flow. Resistance decreased with increasing lung volume. Resistance was compared between air and sulfur hexafluoride which has about the same viscosity as air but a much lower diffusibility in water and a higher diffusibility in fat. Resistances in a single specimen were approximately the same for the two gases indicating that diffusion does not play a significant role in passage of gas across the intersegmental pathway.

13 *Physiology*

PARTITIONING OF RESPIRATORY RESISTANCE IN MAN. Benjamin G. Ferris, Jr., Lionel Opie,* and Jere Mead. Dept. of Physiology, Harvard School of Public Health and Dept. of Med., Peter Bent Brigham Hospital, Boston, Mass.

The resistance of upper airways (UAR) (larynx and mouth) was measured in unanesthetized adult subjects during mouth breathing and compared with total respiratory resistance (TRR) (airways, lungs, and thorax). The pressure difference across the upper airway was measured between a needle inserted into the trachea between the first and second tracheal rings and a similar pressure tap in a tube at the mouth with a cross section comparable to that of the trachea. TRR was measured by superimposing oscillations in pressure on spontaneous breathing and relating these to the resulting oscillations in flow. Four subjects showed higher UAR on inspiration and two were higher on expiration. Average values for UAR and TRR showed no significant difference between inspiration and expiration. The average inspiratory and expiratory resistance of the larynx and mouth was curvilinear and could be expressed by the following formula: $\Delta P = 0.41\dot{V} + 0.48\dot{V}^2$. The resistance of the lower respiratory tract, lungs and thorax, obtained by subtracting UAR from TRR, was found to be linear in the range of flows encountered (0-1.2 lps). The UAR made up 15-20% of the TRR at low flows (0.2-0.4 lps) and was 35-40% at higher flows (1.0-1.2 lps).

14 *Physiology*

MAXIMUM AND MINIMUM AIRWAY PRESSURES AT VARIOUS LUNG VOLUMES IN NORMAL CHILDREN AND ADULTS. C. D. Cook and J. Mead (intr. by J. L. Whittenberger) Children's Hosp. Med. Center, Harvard Med. School and Dept. of Physiology, Harvard School of Public Health.

Maximum positive and negative airway pressures have been measured as a function of lung volume in 43 normal subjects ranging in age from 6 to 47 years. A method requiring only a series of containers of different volumes (1.5-120 liters) and U-tube manometers was used. The subjects breathed into or out of a container as deeply as possible; the pressure change indicated both the maximum pressure and, by application of Boyle's Law, the lung volume change. The plots of maximum pressures as a function of lung volume are similar in shape to those reported by Rahn et al (Am. J. Physiol. 146:161, 1946) but the pressures of comparable subjects on expiration are considerably greater (+60%). Adult males were able to achieve the greatest pressures ranging on expiration from 237 cm. H_2O at 82% of their TLC to 57 cm. H_2O at 30%. On inspiration the adult males' maximum pressures ranged from -133 cm. H_2O at 30% TLC to -38 cm. H_2O at 90%. The three youngest children (between 6 and 9 1/2 yrs.) produced only slightly smaller pressures than the adult males.

15 *Physiology*

MECHANICS OF BREATHING DURING EXERCISE IN MAN. Er Yi Ting (intr. by M. Henry Williams, Jr.) Albert Einstein College of Medicine, New York.

Lung compliance was determined in 9 normal adult male subjects standing at rest and during treadmill exercise. All exercise was performed at 3 mph; the work load was altered by varying the grade from 0 to 20%. Intrapleural pressure was recorded via an intraesophageal balloon. A Fleisch pneumotachograph measured flow rate; the integrated flow signal was utilized for tidal volume. Each compliance measurement was taken as the average of 10 even respiratory cycles. The following alterations were noted during exercise: (1) Compliance fell slightly in 7 of the 9 subjects. (2) The end-tidal esophageal pressure became less negative in all subjects (about 2 cm H_2O). (3) The expiratory reserve volume fell in all subjects (about 500 ml). (4) The vital capacity fell in all (about 450 ml). (5) The mean pulmonary non-elastic resistance was unaltered. (6) The work done on the lung and air column increased as a linear function of treadmill grade. The apparent reduction in lung compliance may have resulted from the increased tidal volume during exercise. The decreased expiratory reserve volume and lessened end-tidal esophageal pressure may both result from altered mechanics of the thoraco-abdominal wall during exercise.

16 *Physiology*

EFFECT OF EXTERNAL IMPEDANCES ON VENTILATORY RESPONSE TO CARBON DIOXIDE. A. B. Gould, Jr.* and W. S. Fowler. Mayo Clin. and Mayo Found., Rochester, Minn.

It is known that when normal persons breathe air through sufficient external resistance to air flow, alveolar pCO_2 is increased and the ventilation rate and ventilatory response to inspired carbon dioxide are reduced. To determine if these changes are related specifically to increased work of breathing, measurement of tidal and minute volume, breathing frequency and end-tidal pCO_2 were made on 8 normal men to compare the effects of 10 minutes of inhalation of 5% carbon dioxide in oxygen (a) without impedance, (b) while breathing through an orifice 5 mm. in diameter, (c) while the trunk was restricted with external elastic belts. Alveolar pCO_2 increased similarly with all procedures. The rate and extent of increase of minute volume were similar without impedance and with restrictive belts, but were decreased with orifice resistance, mainly because frequency did not increase. When breathing of air was resumed, with impedances continued, "undershooting" of alveolar pCO_2 usually occurred within the first 2 minutes with belts, but within the second 2 minutes with orifice resistance. The ventilatory response to inspired carbon dioxide differs when work of breathing is increased by resistance to air flow or by external thoracico-abdominal restriction.

XUM

17 **Physiology**

INSPIRATORY "CHECK VALVE" OBSTRUCTION IN BULLOUS EMPHYSEMA. L. C. Reed*, Akio Furusho*, Thomas H. Snider* and Benjamin M. Lewis. Wayne State U. Coll. Med. and V. A. Hosp., Dearborn, Mich.

Pulmonary cysts or bullae which do not communicate with the bronchi should increase in volume during inspiration and thus press upon and narrow the airway. We have recently studied a 31 year old Negro male who demonstrated such inspiratory "check valving." X-rays showed severe, bilateral, bullous emphysema. Residual volume was reduced (891 ml. STPD) indicating that the cysts did not communicate with the bronchi. Vital capacity was 950 ml. BTPS; maximum breathing capacity, 26 L/Min; diffusing capacity for carbon monoxide, 3.3 ml/mm Hg/min. Compliance was very low (0.02 L./cm H_2O). Inspiratory resistance to air flow was very high, (19.3 cm H_2O/L./sec.) and approximately three times the expiratory resistance (6.4 cm H_2O/L./sec.). The pressure-flow diagram showed a distinctive triangular pattern.

18 **Physiology**

EFFECT OF INHALATION OF CIGARETTE SMOKE AND BRONCHO-ACTIVE AEROSOLS ON AIRWAY CONDUCTANCE. J.A.Nadel*, D.F.Tierney*, and J.H.Comroe,Jr. Cardiovascular Res.Inst.U.of Cal.San Francisco.

We used the body plethysmograph to measure the acute effects of cigarette smoke on airway conductance in 36 healthy subjects. We expressed the measurements as the ratio of airway conductance/thoracic gas volume. After ten inhalations of cigarette smoke, the airway conductance decreased immediately ($p < 0.05$). The average maximal decrease was 50% and lasted approximately one hour. Changes were similar in smokers and non-smokers and were reproducible. Smoking a pipe, cigar, or cigarette without inhaling had no effect on airway conductance. Inhalation of isoproterenol aerosol (0.5%) prevented or reversed the effect of cigarette smoke. In twenty-two patients with cardiopulmonary disease with a wide range of initial airway conductance, cigarette smoking led to changes similar to those in normal subjects. Cigarettes of low and high nicotine content (0.5 mg vs 2.4 mg) caused similar changes in conductance. Inhalation of aerosols of 0.9% NaCl and of nicotine bitrate (2 mg/cc) had no effect. We believe that changes with smoking may be due to inhalation of submicronic particles contained in the smoke and not to the nicotine. The effects of other bronchoconstrictive agents, such as histamine, have been studied to clarify the mechanisms involved. (Supported by the Cal.State Dept.Pub.Health & Tobacco Industries Research Comm.)

19 **Physiology**

A NEW METHOD FOR THE MEASUREMENT OF THE RESISTANCE AND COMPLIANCE OF THE LUNGS AND THORAX IN MAN. Malcolm B. McIlroy, Donald F. Tierney,* and Jay A. Nadel.* Cardiovascular Research Institute, Univ. of Calif. Med. School, San Francisco.

The time constant (RC) of the lungs and thorax is measured from a relaxed expiration. Air is expelled by the elastic recoil P(el) of the lungs and thorax and, if expiration is passive, P(el) is equal to the pressure drop P(resist) across the resistive elements of the system. Thus P(el) = P(resist). If the compliance and resistance of the lungs and thorax are linear in the range of volumes and flows used, $P(el) = V/C$ and $P(resist) = R\dot{V}$. Substituting:- $V/C = R\dot{V}$ or $V/\dot{V} = RC$. Volume and air flow are displayed on the axes of an X-Y recorder and a straight line of slope RC obtained during the latter part of a passive expiration from 1.5 L above resting respiratory level. The procedure is then repeated with a known linear resistance added, and the values of R and C calculated from the two simultaneous equations obtained. The values in normal subjects ranged from 0.04-0.09 L/cmH_2O for compliance, 4.2-8.0 cmH_2O/L/sec for resistance and 0.3-0.5 sec for time constant. When equivalent measurements are made on the lungs using an esophageal balloon, it is found that the time constant of the lungs in normal subjects is equal to the time constant of the thorax. (Supported by USPHS Research Grant H-4029)

20 **Physiology**

RAPID SHIFT OF VENTILATION FROM UNPERFUSED REGIONS OF LUNG. J.W.Severinghaus, E.W.Swenson,* T.N.Finley,* and M.T.Lategola.* Cardiovascular Research Inst., U. of Calif., San Francisco.

We have found that temporary unilateral pulmonary artery occlusion (PAO) causes prompt shift of tidal volume (V_T) away from the unperfused lung in dog and man. Tracheal dividers and self guiding balloon catheters were placed in 16 dogs. Within 5 min. after right PAO, right V_T fell from 55% to 43% of total V_T. From left PAO, left V_T fell from 45% to 32%. This response depends on the fall of P_{CO_2} in the unperfused lung causing bronchoconstriction. It can be prevented by giving 3-6% CO_2 to the lung at the time of occlusion, or by isuprel aerosol, but not by vagotomy. In the unperfused lung, resistance was doubled, and compliance, FRC and anatomic dead space fell. No trapping was found on N_2 washout, nor was shunting past occluded alveoli detectable with the O_2 electrode on release of the occlusion. Therefore we cannot attribute the FRC and compliance fall to occlusion of airways. 1-3 months after left PA ligation in dogs, left lung hypoventilation was no longer related to CO_2. This ventilation distribution mechanism helps explain why pulmonary artery occlusion gives less than predicted increases in ventilation, P_{CO_2} and alveolar dead space. The amount of non-perfused lung is thereby underestimated by measuring CO_2 gradients and alveolar dead space. (Supported by USPHS Research Grants H-3961 and H-4029)

21 **Physiology**

PULMONARY ALVEOLAR STABILITY. John A. Clements. Directorate of Medical Research, Army Chemical Center, Md. (Visiting Scientist, Cardiovascular Research Inst., U.of Cal.Med.Sch., San Francisco.)

Twenty-three human and five rat lungs obtained post mortem were degassed and filled with air or saline for determination of nearly-static pressure-volume (PV) relationships. Saline extracts were prepared from the specimens and examined for their surface film-forming activity. Only minor differences were seen among the saline PV diagrams. Both the volume of air in the alveolar structure of the lungs when deflated to a standard "functional residual pressure" of five cm. of water and the surface-activity of the extracts showed wide variation. When numerical ratios were defined to express these two properties quantitatively, a high degree of correlation was observed between them. The range of correlation was extended by treating the rat lungs with non-ionic detergents. These findings support the hypothesis that the stability of the alveolar structure is dependent upon its surface properties and hence upon intrinsic surface-active material; they permit further refinement of a theory of alveolar statics, considering forces in both the tissue and the surface of the lungs.

(Supported in part by USPHS Research Grant H-4247)

22 **Physiology**

LARYNGEAL REGULATION OF AIRWAY RESISTANCE. Christen Rattenborg. (Intr.by Duncan A. Holaday). Bispebjerg Hospital,Copenhagen, Denmark, and University of Chicago.

Expiratory narrowing of the glottis and ascent of the larynx are correlated movements (Fink et al.). Direct observation snows a greater upward movement of the larynx during oral exhalation than during nasal. Added expiratory resistance reduces the movement. Roentgenography indicates a widening of the laryngeal opening when expiratory resistances are added. The opening is larger during nasal exhalation than during oral. The time constant of the respiratory tract (resistance times compliance) is measured from the expiratory spirogram by fitting to calculated curves. Added resistance prolongs the time constant of a resistance-compliance lung model and of laryngectomized subjects. The majority of normal subjects maintained an unchanged time constant with added resistances of 3.5 and 6.5 cm H_2O/liter/sec. and a few with 9.5 cm H_2O/liter/sec. It is postulated that the larynx constitutes a variable and adjustable resistance, which can compensate for variation in upper airway resistance, and tends to maintain a constant total airway resistance.

238. RESPIRATORY TRACT—Respiration—O_2, CO_2 Transport

1 *Physiology*

VENTILATORY RESPONSES TO CARBON DIOXIDE STRESS IN HYPOTHERMIC DOGS. I. Salzano and F. G. Hall. Duke Univ. Medical Center, Durham, N. C.

Ventilatory responses to carbon dioxide stress during ice-water immersion hypothermia were studied. Stresses were imposed by increasing the CO_2 tension of the inspired gas (4% and 5.5% CO_2 in air) in 8 animals and by I.V. infusion of gaseous CO_2 (67 ml/min for 20 min) in 5 other dogs. Responses to increased CO_2 tension of inspired gas were analyzed by using the ratios of CO_2 inhalation/room air inhalation at 37°C and at 27-28°C. The ratios at normal body temperature for pulmonary ventilation were 1.39 for 4% CO_2 in air and 1.94 for 5.5% CO_2 in air. During hypothermia the ratios were 1.13 and 1.34 for 4% and 5.5% CO_2 in air, respectively. End tidal CO_2 ratios were 1.20 and 1.36 for the two gases during normothermia and 1.28 and 1.51 during hypothermia. Ratios for respiratory rate and maximum inspiratory flow rates were greater than unity when inspired CO_2 gas tension was elevated both during normothermia and hypothermia. Hypothermic animals tolerated CO_2 infusion and responded in a manner qualitatively similar to the normothermic animal. (Supported by Air Force Contract 33(616) 3821.)

2 *Physiology*

AN EVALUATION OF THE RESPIRATORY RESPONSE TO BREATHING 7.5% CO_2 IN AIR AS A TEST OF RESPIRATORY CENTER DEPRESSION. George N. Bedell and Robert S. Rosenberg (intr. by Ralph G. Janes.) Pulmonary Lab., CV Res. Lab., Dept. of Med., College of Med., State Univ. of Iowa, Iowa City, Iowa.

Carbon dioxide has a direct action on the brainstem resulting in increased minute volume of ventilation. As an improved test of the functional competency of the respiratory center, we relate the maximal breathing capacity to ventilatory rate while breathing 7.5% CO_2. A graph of this relationship has been drawn using 6 normal subjects with and without resistance to expiratory airflow artificially added to the expiratory breathing tube to simulate obstructive diseases. The graph was then applied to the data obtained on 135 patients with various diseases. In patients with emphysema, pulmonary fibrosis, bronchiectasis, asthma, polycythemia vera and congenital heart disease, decreased ventilatory response to increased Pa_{CO_2} can be explained on the basis of mechanical obstruction to ventilation. In 11 patients with obesity and in 15 patients with myxedema, all with normal O_2 saturation and Pa_{CO_2}, the mean responses to breathing 7.5% CO_2 were less than would be predicted on the basis of their maximal breathing capacity. Since their Pa_{CO_2} was normal, other factors than respiratory center depression and airway obstruction are implicated.

3 *Physiology*

RELATIONSHIP OF FETAL PLASMA P_{CO_2}, pH AND BICARBONATE TO THE MATERNAL. Jack N. Blechner, Giacomo Meschia and Donald H. Barron. (intr. by Davenport Hooker). Yale Univ. School of Med., New Haven, Conn.

As part of a continuing study of the acid-base balance of fetal sheep and goats the P_{CO_2}, pH and bicarbonate levels of the fetal and maternal plasmas were compared by repeatedly sampling unanesthetized, unrestrained animals through indwelling plastic catheters during periods up to 24 hrs. In animals free from obvious stress the P_{CO_2} in the blood of the umbilical artery exceeds the maternal arterial by approximately 20 mm Hg and the pH is about 0.2 unit less than in the maternal artery, but we observed no regular relationship between the bicarbonate concentrations of mother and fetus. During recovery from stress, whether postoperative or otherwise induced, the fetal-maternal P_{CO_2} gradient remains relatively stable despite wide variations in maternal arterial P_{CO_2}. Maternal infusion of NH_4Cl produces marked and sustained reduction of the bicarbonate in the maternal plasma; however the level in the fetal plasma is virtually unchanged and is independent of the maternal over a 24 hr. period. Our study also demonstrates that the pH of the fetal blood can be less than, equal to, or greater than that of the maternal in some circumstances, and that the pH's of the maternal and fetal bloods may change in opposite directions.

4 *Physiology*

OXYGEN DISSOCIATION CURVES OF THE BLOODS OF PREGNANT SHEEP, FETAL AND NEWBORN LAMBS AT VARIOUS pH's. G. Meschia*, A. Hellegers*, J. Blechner*, S. Wolkoff* and D. H. Barron. Yale Univ. School of Med., New Haven, Conn.

As part of a study on the transfer of respiratory gases across the placenta, oxygen dissociation curves of the bloods of pregnant sheep, fetal and newborn lambs have been prepared at plasma hydrogen-ion concentrations in the physiological range. At pH 7.4, the fetal blood was half saturated at about 16.5 mmHg oxygen pressure. The maternal oxygen dissociation curves could be divided into two groups according to their position: at pH 7.4 the bloods of one group of animals was half saturated with an oxygen pressure of 32 mmHg, that of the other group was saturated at 37 mmHg. A comparison with a similar study on a group of sheep living at 15,000 feet altitude (Barron, Hellegers, Huckabee, Meschia, Metcalfe, Prystowsky, to be published) shows no appreciable differences in the oxygen dissociation curves of the maternal and fetal bloods of the sea level and high altitude animals.

5 *Physiology*

ANALOGUE COMPUTER ANALYSIS OF CO_2 CONCENTRATION CURVES. F. E. Noe, J. Yoshitake and F. E. Greifenstein (intr. by F. A. Morin.) Detroit Receiving Hospital and Wayne State U. College of Med., Detroit, Michigan.

An electronic analogue computer is used to combine the curves from the infrared CO_2 analyzer and from a pneumotachograph to give a flow rate curve of expired CO_2. The method is similar to the method of DuBois, Fowler, Soffer, and Fenn (1952), who used the average expired CO_2 concentration and the volume of flow for small discrete intervals of time to estimate the pulmonary dead space volume. The analogue computer method permits the continuous multiplication of both curves to obtain a CO_2 flow rate curve. The accuracy of the method depends only on 1) the accuracy of the instrumentation, 2) the accuracy with which the time lag between the two curves is measured, and 3) the accuracy of the analogue computer. Integration of the CO_2 flow rate curve gives the volume of expired CO_2. Comparison of the computer CO_2 flow rate curves is used to evaluate pulmonary distribution of air in normal subjects and in abnormal states. (Supported by a grant from the Natl. Insts. of Health.)

6 *Physiology*

IN VIVO INHIBITION OF ERYTHROCYTIC CARBONIC ANHYDRASE IN MAN. Geo. G. Burton*, C.R. Collier, J.D. Hackney* and Carl Bauer*. Dept. of Physiology and Internal Medicine, College of Medical Evangelists, Los Angeles, California.

It has been previously reported (Collier, Fed.Proc. 18:29, 1959) that moderate red cell concentrations (20-30 μg/ml) at a dose of 15-25 mg/kg of acetazoleamide produced maximum interference with CO_2 transport in dogs as evidenced by a ratio of alveolar (A) to arterial (a) PCO_2 of about 0.5. In these studies, normal human subjects were first studied after an oral dose of 10-20 mg/kg. No differences of minute volume, endtidal PCO_2 or other ventilatory parameters were observed after drug while breathing room air or 10% O_2 at rest or breathing 11% O_2 during exercise. There was no difference in the endtidal PCO_2 at 14,000' breathing air. However, an increased ventilatory response to 2 and 4% CO_2 in air was observed after the drug. After a sufficiently large (25-50 mg/kg) intravenous dose, a change similar to that observed in dogs was observed. The maximum drop in A/a ratio ranged from 0.8 - 0.6 at rest and 0.3 during excerise and was reached at a red cell concentration of about 60-80 μg/ml. The observed differences between dogs and humans is probably due to a species difference in carbonic anhydrase as evidenced by the results of 2 different types of in vitro studies.

XUM

7 *Physiology*

GASEOUS EXCHANGE FOLLOWING ACUTE EXPERIMENTAL PULMONARY EMBOLISM IN DOGS. M.Stein, C.E.Forkner,Jr., E.D.Robin, and S.Wessler. Beth Israel and Peter Bent Brigham Hosps. and Harvard Medical School, Boston, Mass. (intr. by A.S.Freedberg)

Endogeneous serum-induced thrombi formed in peripheral veins were released to pulmonary arteries and the resulting respiratory changes recorded. Respiratory rate and minute ventilation rose significantly following pulmonary embolization. The increase in dead space ventilation was disproportionately greater than the augmented alveolar ventilation. In 10 animals with massive embolization, the arterial O_2 saturation decreased from 80% to 75% while in 9 animals with smaller quantities of emboli, the decrease was from 92% to 90%. 8 of 10 animals ventilated with 100% O_2 for 20 minutes following extensive embolization failed to show an increase in arterial O_2 content to the pre-embolic levels obtained with 100% O_2. Significant arterial-alveolar CO_2 tension gradients developed after massive emboli. These derangements in respiratory function were related to the extent of pulmonary embolization. The results of the arterial O_2 saturation studies indicate that emboli of the dogs' own blood induced a perfusion defect consistent with a right to left shunt. These changes in arterial blood O_2 saturation cannot be explained on the basis of pulmonary diffusion or alveolar hypoventilatory defects alone.

8 *Physiology*

OXYGEN USE BY DOG SKELETAL MUSCLE DURING PROGRESSIVE HYPOXIA. Wendell N. Stainsby, Stephen M. Cain* and Arthur B. Otis, Dept. of Physiology, Univ. of Florida, College of Medicine, Gainesville, Florida.

To examine the relationship between oxygen use and arterial P_{O2} in normally circulated mammalian skeletal muscle the venous outflow from the gastrocnemius-plantaris was isolated and metered. The arterial circulation to the muscle group was left intact. Arterial and venous blood samples were drawn and oxygen content measured. Oxygen use was calculated from the Fick Equation. The dogs were exposed to progressive hypoxia by rebreathing room air in a closed circuit. P_{AO2} was measured. As a first approximation P_{aO2} was assumed to be equal to P_{AO2}. P_{vO2} was estimated from *in vivo* dissociation curves constructed from simultaneous measurements of P_{AO2} and S_{aO2}. Oxygen use was constant and therefore independent of P_{aO2} until the P_{aO2} reached approximately 60 mm. Hg. Below this critical value of P_{aO2} oxygen use was reduced as P_{aO2} decreased. The corresponding critical P_{vO2} was approximately 30 mm. Hg. (Supported by a research grant from the Public Health Service, RG 6264.)

9 *Physiology*

OXYGEN DIFFUSING CAPACITY OF TISSUE. A.B.Otis, Univ. of Fla., College of Medicine, Gainesville, Fla.

The problem of estimating the diffusing capacity of a tissue for O_2 is analagous to but apparently even more complex than that for the lung. From such data as are available it appears that the steady state oxygen consumption ($\dot{V}o_2$) of a tissue (resting muscle) is constant over a considerable range of oxygen pressures in the capillary blood perfusing the tissue ($P_{cap.O2}$); but that below some critical $P_{cap.O2}$, $\dot{V}o_2$ falls in an approximately linear relationship with $P_{cap.O2}$. In a steady state, $\dot{V}o_2$ represents both the rate of transfer of O_2 by diffusion and the rate of utilization by oxidative enzymes within the cell. If it is assumed that for the rate of transfer, $\dot{V}o_2 = D(P_{cap.O2} - P_{xO2})$ where D is the diffusing capacity of the tissue and P_{xO2} the partial pressure of O_2 at the site of utilization; and that for the rate of utilization $Vo_2 = K P_{xO2}$, where K is a constant; then it follows that $d\dot{V}o_2/dP_{cap.O2} = K D/K+D$. Thus changes in $\dot{V}o_2$ with changes in $P_{cap.O2}$ are dependent on both diffusing capacity and properties of the oxidative enzymes. (Supported by Contract AF 41 (657) 102 with School of Aviation Medicine.)

10 *Physiology*

THE EFFECT OF ACIDOSIS ON CEREBROSPINAL FLUID (CSF) AND ITS CORRECTION BY THAM. Serge J. Dos* and Gabriel G. Nahas (intr. by William M. Manger). Dept. of Anesthesiology, Coll. of Physicians & Surgeons, Columbia Univ., New York, N. Y.

Respiratory acidosis was produced in 8 dogs by maintaining them in apneic oxygenation (AO) for 35 minutes: CSF pressure (CSFP) rose from 61 to 147 cm H_2O, arterial pH fell from 7.60 to 6.87 and CSF pH from 7.54 to 6.93; $paCO_2$ rose from 21 to 157 mm Hg and CSF pCO_2 from 31 to 117. During a second period of AO 4 dogs received IV .33 mM/Kg/min of .3 M trishydroxymethylaminomethane (THAM). CSFP did not change, arterial and CSF pH stayed within .1 pH unit of control, while arterial and CSF pCO_2 rose respectively from 24 to 58 and from 33 to 51 mm Hg. Four other dogs were given THAM titrated to pH 7.40 with HCl. The increase in CSFP was half of that of the control hypercapnic period, while blood and CSF pH and pCO_2 changes were the same, indicating that THAM acts on CSFP through both its buffering and osmotic properties. In 5 additional dogs mechanically ventilated a fixed acidosis was produced by an IV infusion of .5 M HCl: after 40 minutes, CSFP increased from 41 to 91 cm H_2O; arterial pH fell from 7.40 to 6.69 and CSF pH from 7.46 to 7.12; $paCO_2$ rose from 33 to 43 mm Hg and CSF pCO_2 from 33 to 38. In acutely induced acidosis, changes in CSFP are best correlated with changes in CSF pH.

11 *Physiology*

Aerobic Capacity in Relation to Body Size and Composition
D.Cardus*, T.P.K.Lim, E.C.Anderson*, J. Howarth* and U.C.Luft
Lovelace Foundation, Albuquerque, and Los Alamos Scientific Laboratories, New Mexico.

Total oxygen intake during exercise approaching aerobic capacity is dependent upon: 1. The active tissue mass. 2. Cellular metabolism. 3. The efficiency of circulation and respiration. The physical condition of an individual depends primarily on items 2 and 3 and could be determined more precisely if the active tissue mass were known so that variations attributable to this quantity could be taken into account. For this purpose six different parameters were tested as to their value as indices of active tissue mass: body weight, surface area, lean body mass (density method), total body water (Tritium), total potassium (K^{40}) and circulating hemoglobin (carbon monoxide). The oxygen intake of 60 healthy, untrained men aged 28-38, measured during the final minute of exercise on a bicycle ergometer at a work load where the heart rate reached 180, was correlated with each of these parameters. There was a statistically significant correlation between all six parameters and "final oxygen intake". However, that with lean body mass was the best, followed by total body water which was also highly significant and notably better than with body surface area, body weight, potassium and circulating hemoglobin.

12 *Physiology*

INTRACELLULAR OXYGEN TENSION ESTIMATED FROM THE GLOW OF FIREFLIES. Arthur B. DuBois and George Polgar*. Graduate School of Medicine, Univ. of Pa., Phila., Pa.

Since most of the O_2 uptake of cells is by their minute mitochondria, P_{O_2} gradients should exist between the cell surface and the mitochondrial surface. The diffusion equation would be: $P_{O_2}(1)-P_{O_2}(2) = \frac{Qm O_2}{4\pi\alpha D} \int_{r_1}^{r_2} \frac{dr}{r^2}$, where $Qm O_2$ is the rate of O_2 uptake of a mitochondrium of radius r_1 surrounded by a spherule of cytoplasm of radius r_2, α the solubility, and D the diffusion coefficient of O_2. A large tension gradient has been calculated using this equation, and also found by comparing the range of P_{O_2} that influences the steady glow of fireflies *in vivo* with the range of P_{O_2} which affects the glow of light organ extracts *in vitro*. An O_2 electrode was used to estimate interstitial O_2 tension. After exposure to propylhexedrine vapor, Photuris emitted a steady glow which was minimum at a P_{O_2} of 2 mm Hg, and maximum at 2000 mm Hg, ambient. Interstitial P_{O_2} was about 250 mm Hg when ambient was 750 mm Hg. By contrast, maximum steady glow of cellular extracts *in vitro* occurs at 8 mm Hg, minimum at about 0.002 mm Hg (J.W. Hastings, et al, J. Cell. Comp. Physiol. 42:137, 1953). It was concluded that the oxygen tension inside cells may be appreciably less than outside. This gradient may play an important role in the glow of fireflies and in other life processes affected or regulated by O_2.

13 **Physiology**

PARTITION OF CO AND O_2 BETWEEN ALVEOLAR GAS AND THE HEMOGLOBIN OF ARTERIAL BLOOD. W.S. Blakemore, G.K. Danielson,* and R. E. FORSTER. Harrison Dept. Surg. Res., Sch. of Med. and Dept. of Physiology, Graduate School of Med., Univ. of Pa., Phila., Pa.

The in vivo competition of CO and O_2 for intracellular hemoglobin was evaluated in 16 subjects according to the formula, a constant (M) = (COHb x O_2 tension)/(O_2 Hb x CO tension) of arterial blood. The subjects breathed O_2 in an open circuit for 8 minutes to wash the N_2 from their lungs and then rebreathed on a closed circuit containing O_2 with a CO_2 absorber for 15 min. At the end of this period a sample of arterial blood was obtained and analyzed for CO_2, O_2, CO, pH and Hb, and gas samples were obtained from the rebreathing bag and analyzed for O_2, CO_2, CO, and N_2. Stalex hopcalite (minimal detectable volume 0.0001 ml.) and infrared (minimal detectable volume 0.0005 ml.) analyzers were used to measure blood COHb and gas CO concentration. Van Slyke and Scholander apparatuses were used to determine the blood O_2Hb and gas O_2 concentrations respectively. It was assumed that the ratio of blood O_2 to CO tension equaled that of the gas in the rebreathing bag. M was 270,SD ± 14. In patients 30 ml. of CO was added to the rebreathing circuit and after 30 minutes, M was 265,SD ± 18.

14 **Physiology**

THE ALVEOLO URINARY N_2 PARTIAL PRESSURE DIFFERENCE COMPARED TO OTHER MEASURES OF THE DISTRIBUTION OF VENTILATION AND PERFUSION WITHIN THE LUNG. W. A. Briscoe and H. P. Gurtner*. Department of Medicine, Columbia Univ., New York, N.Y.

In 10 normal subjects the partial pressure of N_2 dissolved in the urine at body temperature exceeded that in the mixed expired alveolar gas by 3.3 mm Hg. with a standard deviation of 2.3 mm. In 10 patients with severe chronic pulmonary emphysema the excess pressure averaged 19.8 mm with a standard deviation of 5.3 mm Hg. This Alveolo Urinary N_2 partial pressure difference reflects the inequality of ventilation perfusion relationships in the lung (Rahn and Klocke, Fed.Proc. 18,489,1959). Three other methods are now available for assessing the degree of this inequality. These depend on: 1) arterial O_2 Sat., 2) slowly eliminated Kr^{85}, 3) arterial Kr^{85}. The results of these methods were compared with the Alveolo Urinary N_2 partial pressure difference in the patients with emphysema. In some studies there were discrepancies, but on the whole the Alveolo Urinary N_2 partial pressure difference is of the order of magnitude expected from studies of the distribution of ventilation and the distribution of perfusion by the other methods.

15 **Physiology**

THE P_{O_2} AND P_{CO_2} OF LYMPH. E.H.Bergofsky*, J.H.Jacobson II* and A.P.Fishman. Columbia Univ.,N.Y.

The P_{O_2} and P_{CO_2} of lymph from the cysterna chyli and the thoracic, femoral and cervical ducts were determined in 8 anesthetized dogs. The P_{O_2} was measured by the platinum-silver polarograph electrode. The P_{CO_2} was calculated from the CO_2 content and pH values. Lymph from all these sites had a lower P_{O_2} (9±6 mm Hg) and a higher P_{CO_2} (55±7 mm Hg) than did regional or mixed venous blood (P_{O_2}=43±12 mm Hg, P_{CO_2}=44±6 mm Hg). After 45 minutes of mechanical hyperventilation, the P_{CO_2} of lymph and venous blood decreased proportionally, with no change in the P_{O_2} of lymph. Breathing pure O_2 for one hour failed to eliminate the ΔP_{O_2} between lymph and venous blood. The differences between the tensions of lymph and venous blood were shown not to arise from either: 1) O_2 consumption by lymphocytes or lymphatic vessels, 2) differences in the pK_a of H_2CO_3 of lymph and blood, or 3) failure of lymph and blood tensions to equilibrate during the period of observation. The data suggest that the tissues from which the lymph is derived, normally function at extremely low O_2 tensions.

16 **Physiology**

RESPIRATORY ADAPTATION TO CHRONIC CO_2 EXPOSURE. K.E.Schaefer, G. Nichols* and C.R.Carey*. U.S.Naval Med. Research Lab., New London, Conn. and Biochemistry Dept., Harvard Med. School, Boston, Mass.

In a previous paper it was reported that subjects exposed to 3% CO_2 in 21% O_2 for prolonged periods showed a characteristic respiratory adaptation which consisted, among other factors, in a reduction of the normal ventilatory response to increased concentration of CO_2, 5% and 7% CO_2. (Schaefer, K. E., Pflueg. Arch., 251, 689, 1949). For the present study 23 subjects were exposed to 1.5% CO_2 in 21% O_2 for 42 days. Respiratory minute volume and alveolar CO_2 tension were found increased throughout the exposure but no significant changes in vital capacity, inspiratory and expiratory reserve, or tidal volume, were observed. A slight respiratory acidosis was present during the first 23 days of exposure as indicated in the lowering of pH, which was associated with a decreased CO_2 excretion in the expired air and in the urine. A phase of compensated respiratory acidosis followed, during which the pH, as well as the CO_2 excretion, returned to initial levels. After transition to air the CO_2 excretion in expired air and urine reached a peak and returned to normal values within a week. At the end of the exposure to 1.5% CO_2 a significant depression of the ventilatory response to 5% CO_2 was found, similar to the results under 3% CO_2, previously reported.

17 **Physiology**

TECHNIQUE AND EXPERIENCES WITH GAS CHROMATOGRAPHY IN BLOOD GAS ANALYSIS. Bernard Fisher, M.D. and B. W. Taylor, B. S. *. Laboratory of Surgical Research, University of Pittsburgh Medical School, Pittsburgh, Pa.

While gas chromatography has been widely used in industry, it has received little attention as a technique in biological analysis. Using a unique reaction chamber as part of a chromatographic system in which gases are extracted chemically from as little as 0.1 ml of whole blood, oxygen and CO_2 content may be determined. Complete separation and detection of the gases in the sample are effected by the chromatographic system and the proportions of each are recorded consecutively with a strip-chart recorder within 5 minutes.

Repetitive analysis of individual samples demonstrate a variation of less than 1% for CO_2 and less than 1.5% for oxygen. Values obtained with this method correlate with the manometric values (Van Slyke) within ± 2 vol. %, assuming that the manometric value is a standard and accurate one. Examples of the application of this method of analysis in animal and human investigations will be presented. (Supported by the Fisher Scientific Co., Pittsburgh, Pa.)

18 **Physiology**

CARBON DIOXIDE BALANCE STUDIES IN NORMAL AND ACIDOTIC DOGS TREATED WITH ORGANIC BUFFER (THAM). Felice Manfredi*, Richard M. Portwood, and Herbert O. Sieker. (Intro. by E.A. Stead,Jr.) Dept. of Med., Duke Univ. Med. Center, Durham, N.C.

Organic buffers are now used in treatment of acute and chronic respiratory acidosis. This study was designed to elucidate the mechanism of action. Infusions of 500 ml. of 0.3 M organic buffer (THAM) were given over 1 hour periods to 16 anesthetized dogs breathing room air (Group 1) or made hypercapnic with inhalation of 6% CO_2 (Group 2). Blood and urine pH, CO_2, Na, K, and Cl, and expired CO_2 were determined before, during, and after THAM infusion. Mean data are tabulated.

		Blood	Urine	Expired Air
Group	pH	CO_2 (mM/L)	CO_2 (mM/min)	CO_2 (mM/min)
before	7.297	15.53	.006	35.4
1.during	7.524	19.67	.114	30.7
after	7.532	22.42	.151	30.0
before	7.149	22.45	.006	21.8
2.during	7.287	27.96	.083	24.1
after	7.222	29.62	.122	31.0

No other significant electrolyte change was observed. These data suggest that THAM rapidly corrects respiratory acidosis by influencing acid-base balance 1) as a chemical buffer and 2) by enhancing CO_2 excretion.

239. RESPIRATORY TRACT–
Respiration–Pulmonary Diffusion

1 *Physiology*

PULMONARY CAPILLARY BLOOD VOLUME AND MEMBRANE DIFFUSION COMPONENT ON EXERCISE. D. V. Bates, C. J. Varvis,* R. E. Donevan,* and R. V. Christie. Joint Cardiorespiratory Service, Royal Victoria Hospital and Montreal Children's Hospital, Mtl., Que.

By measuring the lung diffusing capacity for CO (D_LCO) at differing alveolar oxygen tensions, it is possible to compute the pulmonary capillary blood volume (Vc) and membrane diffusion components (Dm) in subjects with normal intrapulmonary gas distribution making use of the kinetic data of Forster and Roughton. These components have been measured during steady state exercise in 14 normal subjects and 20 patients. The conclusions are as follows: 1.In normal subjects at 3 mph on the flat, Vc averages 140 ml. SD±12 ml. 2.Dm averages 51 with an SD of± 10 ml.CO/min/mmHg. under the same conditions. 3.Removal of parts of the lung is followed by a proportionate drop in Dm. 4.Pulmonary fibrosis is invariably characterized by reduction of Dm in relation to lung volume, and also usually of Vc though this is variable. 5.Conditions with an abnormally high pulmonary flow are accompanied by a normal and not an elevated Vc. 6.Mitral stenosis appears to be often characterized by a high Vc and low Dm. In one patient after a successful valvotomy these values returned to normal, but this reciprocal change was not reflected by any significant change in D_L. (Supported by N.R.C. Grant MT-746)

2 *Physiology*

ALVEOLAR-ARTERIAL OXYGEN GRADIENT IN DOG AT ALTITUDE. Ferdinand Kreuzer. Dept. of Physiol., Dartmouth Med. School, Hanover, N.H.

The effect of 5 days at an altitude of 14,300 ft. on the alveolar-arterial O_2 gradient ($AaDO_2$) in the anesthetized dog with constant ventilation was studied on 3 levels of oxygenation (sea level: 11% O_2, air, 100% O_2; altitude: air, 40% O_2, 100% O_2). The alveolar samples were analyzed for alveolar O_2 and CO_2 pressures (Scholander), the arterial samples for arterial O_2 pressures (polarograph). The average values of $AaDO_2$ for the 3-resp. levels of oxygenation were: sea level: 8.6, 23.4 and 168.5 mmHg (last figure corresponding to a shunt of 12% of cardiac output); altitude: 3.3, 17.0 and 58.0 mm Hg (last figure corresponding to a shunt of 4%). The $AaDO_2$ with air at altitude was lower than with air at sea level and also lower than with 11% O_2 at sea level. The most striking effect was a threefold decrease of $AaDO_2$ with 100% O_2 at altitude as compared with sea level. An explanation of the 2 effects was not possible from these data, but it might be, on the basis of available evidence, that the effect with low O_2 might be due to an increase of the pulmonary diffusing capacity at altitude, and the difference with 100% O_2 as an expression of a decreased true venous admixture might be connected with the epinephrine release at altitude. (Supported by grants from Nat. Heart Inst. (H-2830-C2), N.H. Heart Assoc., and Nat. Science Foundation.)

3 *Physiology*

REFLEX STIMULATION OF PULMONARY VENTILATION BY MIXED VENOUS Pco2. R.E. Dutton*, Som Nath*, and R.L. Riley. University of Puebla,Mexico, and Johns Hopkins University, Baltimore.

Mixed venous Pco2 rises significantly during exercise in contrast to arterial Pco2. During exercise and during inhalation of CO2 mixtures at rest, mixed venous Pco2 was estimated using a rebreathing technique. The relationship between mixed venous Pco2 and ventilation was found to be approximately the same in both circumstances. Furthermore, the latent period before ventilation began to increase after inhaling CO2 or starting to exercise was usually 15 seconds or more. A time lag of this order would be expected before a significant elevation of mixed venous Pco2 occurred,because of the solubility of CO2 in tissue fluids. These findings are consistent with the hypothesis that the increase in ventilation with exercise or CO2 breathing is caused by a chemoreflex, the receptors of which are sensitive to the Pco2 of the mixed venous blood. Studies designed to determine more precisely the location of the receptors are as yet inconclusive. (Supported by Navy,Air Force and Public Health Service.)

4 *Physiology*

EFFECT OF PRESSURE SUIT INFLATION OVER THE LOWER HALF OF THE BODY ON BREATH HOLDING PULMONARY DIFFUSING CAPACITY FOR CO(D_L). J.C. Ross, G.D. Ley, and T.J. Lord, (Intr. by J.B. Hickam), Ind. Univ. Sch. of Medicine and V.A. Hospital, Indianapolis, Ind.

Increase in pulmonary capillary blood volume(V_c) should increase D_L. Others have reported that G-suit inflation did not increase D_L, suggesting V_c did not increase. Central venous pressure(CVP) was not measured, however, and since, in our hands, CVP is not always increased by suit inflation, this subject was re-investigated with CVP measured by catheter in the superior cava and with a study of the effect of alveolar volume(V_A) and Valsalva maneuver on D_L during suit inflation. D_L was determined in seated and supine subjects at rest, with a Valsalva, with G-suit inflation, and with Valsalva during suit inflation. Six of 7 supine subjects had increased D_L with suit inflation at small and large V_A. Mean D_L at rest(mean V_A 3.1 and 4.8 L.) was 31.1 and 38.8 and increased to 33.4(V_A 3.1 L.) ($p<.05$) and 43.6(V_A 4.6 L.)($p<.005$) when mean CVP was raised 14 mm.Hg. In 4 subjects at V_A of 3.1 L., D_L was 31.2, 32.6, & 31.3 and at V_A of 4.6 L., D_L was 38.7, 42.0, & 33.3 at rest, with suit inflation, and with a Valsalva during suit inflation respectively. All 5 subjects who were studied seated had increases in D_L with suit inflation at small and large V_A. Mean D_L at rest (mean V_A 3.4 and 4.7 L.) was 30.8 and 37.0 and increased to 37.1(V_A 3.3 L.)($p<.005$) and 42.7(V_A 4.3 L.)(p NS) with mean CVP increases of 24 and 28 mm.Hg respectively.

5 *Physiology*

INFLUENCE OF RED CELL MEMBRANE ON O_2 DIFFUSION. William Z. Yahr and Ferdinand Kreuzer. (intr. by R.E. Forster). Dept. of Physiol., Dartmouth Med. School, Hanover, N.H.

The available evidence on this problem is still conflicting. In order to evaluate this influence, in vitro experiments were performed on human blood at 37°C. Centrifuged blood samples were divided into two portions, one containing packed red cells and the other consisting of hemolyzed blood representing a highly concentrated Hb concentration as found in the red cells (30-35g%). Since both media had the same Hb concentration, the only difference between them for the O_2 propagation (simultaneous diffusion and chemical reaction of O_2 with Hb) consisted in the presence of the red cell membrane in the case of the packed red cells. Both media were analyzed with a recent modification of the thin layer technique (Helv. Physiol. Acta 9:244, 1951), i.e. they were spread out on a glass plate in thin horizontal layers 100-200 u thick. The light transmission changes resulting from the uptake of pure O_2 by these media were continuously recorded. From such tracings, the one third and one half saturation times were evaluated and compared. No significant difference was found. It was concluded that the red cell membrane does not seem to exert an essential impediment to the O_2 propagation. (Supported by grants from Nat. Heart Inst., N.H. Heart Assoc., Nat. Found. Infantile Paralysis, and Lederle Med. Stud. Research Fellowship).

6 *Physiology*

THE EFFECT OF RESPIRATORY MANEUVERS ON THE UPTAKE OF CARBON MONOXIDE AND CALCULATED DIFFUSING CAPACITY DURING BREATHHOLDING. Theodore C. Smith and John Rankin. (intr. by O. S. Orth.) University of Wisconsin Medical School, Madison 6, Wisconsin.

One criticism of the single breath method for measuring Dco is the change in hemodynamics associated with deep inspiration and subsequent breathholding. To assess this the changes were accentuated by Valsalva maneuvers (ca. 60 mm Hg) and by Muller maneuvers (20-25 mm Hg) during breathholding of 5 to 20 seconds. Measurements were made in 5 healthy subjects at O_2 tensions of approximately 120 and 570 mm Hg. Dco in ml/min/mm Hg was calculated using ambient rather than alveolar pressure.

		5 sec.	10 sec.	15 sec.	20 sec.
pO_2 approx. 570 mm Hg	Muller	15.4	17.5	16.4	17.2
	Normal	18.7	18.8	18.2	18.4
	Valsalva	21.9	17.0	16.3	14.7
pO_2 approx. 120 mm Hg	Muller	33.2	33.3	33.2	32.6
	Normal	34.9	33.3	32.4	31.9
	Valsalva	37.4	30.9	29.9	29.0

Rapid inspiration produces a transient increase in venous return and pulmonary capillary volume, increasing gas uptake. Changes in pressure gradient of the diffusing gas due to these maneuvers have the opposite effect. D_{CO} calculated without regard to these changes has slightly greater variance, but is not significantly changed throughout the first 15 seconds.

7 *Physiology*

DIFFUSION OF OXYGEN INTO RED BLOOD CELLS. N.C.Staub (intr. by Ralph Kellogg). Cardiovascular Research Institute, University of California, San Francisco.

The process of O_2 uptake by erythrocytes involves diffusion across the cell membrane, through the cell interior and, finally, chemical reaction with the hemoglobin molecules. The process of physical diffusion has never been measured directly, but only inferred from the difference between the rate of O_2 uptake in dilute solutions of hemoglobin and in intact red cells. Using a rapid response O_2 electrode and a continuous flow rapid reaction apparatus, I have determined the time course of oxygen diffusion into sheep red blood cells in which the hemoglobin has been inactivated by prior saturation with nitric oxide. The measured rate of diffusion of O_2 is very rapid, the half time for these sheep cells being about two milliseconds. Using available data on gas diffusion in concentrated hemoglobin solutions and simplifications of red cell geometry, the diffusion coefficient of the red cell membrane can be estimated. It is very low, of the order of 10^{-7} cm^2/sec. However, because the membrane is so thin, the permeability of the membrane is about the same as that of the cell interior.

(Supported by ONR Nonr - 222(55))

8 *Pharmacology*

THE INFLUENCE OF BODY POSITION ON THE RATE OF UPTAKE OF CO BY THE HUMAN LUNG. Charles Votava Jr., Alan S. Keitt, Michael McDonald, Theodore C. Smith and John Rankin (intr. by Charles W. Crumpton.) University of Wisconsin Medical School, Madison, Wisconsin.

The diffusing capacity of the lung (D_L) was measured by the CO-He breathholding technique in 6 healthy subjects in several different positions at an oxygen tension of approximately 120 mm Hg. D_L was maximum when the subject was supine and decreased an average 11.5% when the subject sat up, and 10.5% when he turned on his left side. The measurements were repeated at an oxygen tension of approximately 600 mm Hg and the diffusing capacity of the pulmonary membrane (D_M) and pulmonary capillary blood volume (V_c) calculated by the method of Roughton and Forster. Compared with the supine position V_c decreased on average 12.6% when sitting and 15.4% when lying on the left side. D_M decreased proportionately. These findings were observed consistently at all breathholding intervals up to 30 seconds. Separate measurements of D_L in each lung during bronchospirometry suggested that the resting pulmonary capillary volume is maximum in the supine position and that in other positions some of the capillaries in the upper parts of the lung are either empty or only partially filled with blood.

240. RESPIRATORY TRACT—Respiration—Tissue

1 *Physiology*

AN ELECTRODE FOR MEASUREMENT OF TRUE OXYGEN PARTIAL PRESSURE IN BIOLOGICAL ENVIRONMENTS. Albert F. Kelso (intr. by Price E. Thomas) Department of Physiology, College of Osteopathy, Chicago, Illinois.

Tests of an isolated electrolysis cell with controlling electronic circuits indicate it is possible to measure true oxygen partial pressure at normal temperature and pressure independent of the system in which the measurements are made. A teflon membrane permeable to oxygen but not to solvents isolates the electrolysis cell. The electronic circuits balance out the capacitance current and time the polarization and measuring intervals. Measurement of polarographic diffusion current is made before diffusion reaches the surface of the electrode. Thus, the electrode is insensitive to the nature and conditions, such as movement, of the system being measured. The use of reversible oxygen reactions appears to improve the characteristics of the electrode, and several reactions are being investigated. Sensitivity to minimum pO_2 is the same as for other oxygen electrodes. The response time and time to equilibrium depends upon the ratio of effective membrane area to volume of the electrolyte and oxygen permeability of the membrane. Electrodes used to date have a diameter of several millimeters, however, an electrode 0.2 mm diameter and 5 mm length is being developed.

2 *Pathology*

PATHOLOGICAL AND PHYSIOLOGICAL STUDIES OF EXPERIMENTALLY PRODUCED "CYSTIC" LESIONS IN THE LUNGS OF RABBITS. J. Kleinerman, E. Wolinsky*, G.W. Wright, E. Chester*, W. Harold*, and A. Segal*, St. Luke's and Met. Gen. Hosps., Cleveland, O.

Cystic or "bullous" lesions have been produced in the lung of rabbits by intratracheal inoculation of tubercle bacilli, followed by antituberculous therapy. The purpose of this experiment was to study the pathogenesis of these lesions and determine the associated physiologic abnormalities. 25 rabbits were injected intratracheally with tubercle bacilli. Progression of lesions was followed by X-rays. Treatment with isoniazid instituted when moderate or extensive infiltrate was observed. Animals were sacrificed at regular intervals prior to and after therapy was started. Presacrifice physiologic studies were done. Lungs were studied histologically by conventional methods.

Seven rabbits developed the cystic lesions. The physiologic data did not vary consistently from normal controls. Early lesions had thick cyst walls; these were thinned in older lesions. Vinylite injections demonstrated patent, narrowed bronchial communications. Granulomatous and caseous lesions present early, disappeared after therapy. It is postulated that caseous necrosis and evacuation of this material are the processes basic to production of these lesions. Healing produced smooth walled lesions resembling cysts or bullae.

3 *Pathology*

THE ISOLATION AND PHYSICAL PROPERTIES OF SINGLE ELASTIC FIBERS. Robert W. Carton and John Dainauskas (intr. by George M. Hass). Depts. of Path. and Med., Presbyterian-St. Luke's Hospital, Chicago, Illinois.

Single elastic fibers measuring approximately 9 microns in diameter and 200 to 600 microns in length have been dissected free from the ligamentum nuchae of cattle. Under a dissecting microscope the stretch in these fibers in response to applied loads can be observed and measured. Force-deformation curves for 5 such fibers from separate oxen were constructed and were found to show uniform elastic behavior. Hence the elasticity of small units of elastic tissue can be measured. Three fibers subjected to 88% formic acid at 45° for 20 hours showed similar behavior. These single elastic fibers showed a different pattern of elastic behavior from that of gross strips of ligamentum nuchae from the same cattle, implying that the elastic properties of elastic tissue networks can not merely be represented as the sum of the behavior of the individual component fibers.

241. STRESS

1 *Physiology*

PERFORMANCE OF RATS IN STANDARDIZED TREADMILL TESTS. D. C. Jones, D. J. Kimeldorf, and G. K. Osborn. U. S. Naval Radiological Defense Laboratory, San Francisco, Calif.

Standardized methods for testing the performance ability of the rat on a motor-driven treadmill have been developed. It was found that rats are able to perform satisfactorily at treadmill speeds up to 80 ft/min. Endurance times vary directly with experience and inversely with speed and/or angle of inclination of the treadmill. Body weight patterns indicate that repeated exercise to exhaustion at an intermediate speed (40 ft/min) is more stressful than at a lower speed (30 ft/min) for longer periods of time or at higher speeds (60,80 ft/min) for shorter durations of time. Remote recordings of heart rate and deep body temperature at intervals before, during and after one hour of exercise have provided reliable criteria of the rat's physiologic response to exercise. Heart rate increases dramatically during the first few minutes of exercise, decreases slightly during the remainder of the exercise period, and then falls rapidly to resting values upon cessation of exercise. Body temperature follows a similar pattern, although the increase during exercise and the return to resting values after exercise occur more slowly. In contrast to man, where doubling of the heart rate during exercise is common, vigorous exercise increases the heart rate by approximately 25% in the rat.

XUM

2 *Physiology*

EFFECT OF GUANYLIC ACID ON STRESS RESPONSES. E. R. Raney and J. Kabal (intr. by J. C. Rose.) Dept. of Physiol., Georgetown Univ. School of Med., Washington, D.C.

In the rat, stress induces a rise in plasma non-esterified fatty acids (NEFA). Adrenalectomy aggravates this response. In fed rats, daytime NEFA levels averaged 0.686 mEq./L. After a 16 hr. fast these values were elevated to 1.113 mEq./L. The adrenalectomized rat after a 16 hr. fast with access to saline has NEFA levels of 1.550 mEq./L. and an elevated hematocrit. If normal and adrenalectomized rats are pretreated with daily injections of 10 mgm/kg guanylic acid for 5 days and then fasted for 16 hrs. NEFA levels are not elevated over fed levels. The application of hind limb tourniquets to intact rats elevates NEFA levels to 1.616 mEq./L. Pretreatment with guanylic acid prevents this response. 5 hrs. of tourniquet produces 100% mortality in adrenalectomized rats within 2-3 hrs. after removal of the tourniquet. Pretreatment with guanylic acid significantly extends the survival time of such animals. Since guanosine derivatives are found in unusually high concentration in lymphatic tissue, involution of this tissue during stress may release these compounds. A physiological role of the nucleotides in the stress response is thus suggested. (Supported by a grant from the USPHS. Inst. of Arthritis and Metabolic Diseases. A-2307.)

3 *Pathology*

THE EFFECT OF AUDIOGENIC STRESS UPON THE GROWTH OF METHYLCHOLANTHRENE INDUCED TUMORS IN MICE. L. W. SMITH AND F. LAZERE*, WALDEMAR MEDICAL RESEARCH FOUNDATION, PORT WASHINGTON, NEW YORK.

MICE FROM TWO STRAINS, (A/JAX AND DBA), WERE INJECTED SUBCUTANEOUSLY WITH METHYLCHOLANTHRENE, IN VARYING DOSAGES, FOLLOWING AN INITIAL PERIOD DURING WHICH THEY WERE SUBJECTED TO SUBCONVULSIVE AUDIOGENIC STRESS. SUBSEQUENTLY STRESS WAS CONTINUED IN A RANDOM PATTERN UNTIL THE DEVELOPMENT OF TUMOR WAS FIRST NOTED, AND CARRIED ON UNTIL THE DEATH OF THE ANIMAL. MARKED DIFFERENCES IN THE LATENT PERIOD WERE NOTED IN THE TWO STRAINS, AS WELL AS A DELAY IN THE ONSET OF TUMOR FORMATION AND IN THE TIME OF DEATH IN THE STRESS TREATED ANIMALS IN BOTH STRAINS. DEPILATION AND OCCASIONAL PAPILLOMA FORMATION LIKEWISE WERE SEEN EARLIER AND MORE FREQUENTLY AS PRECURSORS OF ACTUAL TUMOR DEVELOPMENT IN THE A/JAX STRAIN AS COMPARED WITH THE DBA/1 MICE. THERE WERE A FEW ANIMALS, MOST OF THEM SUBJECTED TO STRESS, IN BOTH STRAINS WHICH DID NOT DEVELOP TUMORS WITHIN THE USUAL SEVEN MONTH EXPERIMENTAL PERIOD, AND DID NOT DIE UNTIL AS LATE AS THE TENTH OR ELEVENTH MONTH. STRESS CONTINUED TO BE APPLIED TO THE EXPERIMENTAL ANIMALS DURING SUCH PERIODS.

242. TISSUE CULTURE

1 *Physiology*

HORMONAL REQUIREMENTS OF GUINEA PIG MAMMARY TISSUE IN VITRO. George C. Gerritsen (intr. by Lester F. Wolterink).

Tissue culture techniques provide an excellent method to evaluate the hormonal requirements of individual tissues by eliminating systemic influences upon the particular tissue under study. Mammary tissue was cultured by the Chen adaptation of Fell's method. Various combinations and levels of hormones were added to the synthetic medium to evaluate their effect on the performance of the tissue in vitro. Levels of 140 μg prolactin, 8 μg hydrocortisone and 70 μg insulin per ml of medium were found to be most effective in maintaining the tissue in a nonsecretory state. Levels of 240 μg prolactin, 16 μg hydrocortisone, and 140 μg insulin per ml of medium were found to be most effective in maintaining the tissue in an active secretory state. The tissues were maintained in culture for 6 days, after which they were examined by standard histological techniques. When mammary tissue was removed from each guinea pig to provide explants for tissue culture, additional mammary tissue was removed for control purposes. The results show that guinea pig mammary tissue can be maintained in vitro with hormones, and provides a good technique for hormonal and biochemical studies on the isolated living mammary gland. (Predoctoral Fellow, National Cancer Institute, USPHS; also supported in part by NIH grant C-3448 to J. Meites.)

2 *Physiology*

NUCLEAR AND CYTOPLASMIC RELATIONSHIPS BETWEEN NORMAL AND MALIGNANT TISSUE CULTURES. H.J.Phillips, R.V.Andrews* and V. Skank,* Creighton Univ. Sch. of Med., Omaha, Nebraska.

The objective was to compare nucleocytoplasmic ratio, nuclear size and water content of tissue cultured cell strains derived from normal and malignant tissue. When tissue cultures are trypsinized and made into a suspension in 0.9% NaCl the live cells become spherical for a short time. If they are photographed on a hemacytometer the diameter of cells and their nuclei can be accurately measured from photographs. Ordinary light microscopy and a Polaroid camera were used. Only established tissue culture cell strains were used; primary explants were not included in the study. Eleven strains of cells derived from normal tissues were compared with nine strains derived from malignant tumors. About 75 measurements for each characteristic were made for each cell strain. No consistant difference was found between the groups. Cancer cells "in vivo" have been reported to have a higher nucleocytoplasmic ratio, greater variation in nuclear size and more water than normal cells. Therefore, one must conclude that tissue cultures adapt to uniform "in vitro" conditions and in so doing take on similar physical characteristics. (Aided by Natl. Cancer Inst. and Nebr. Heart Assoc.)

3 *Biochemistry*

PROTEIN METABOLISM IN WALKER CARCINOMA 256 CELLS IN TISSUE CULTURE. Morris Green* and Leon L. Miller. Department of Radiation Biology, Univ. of Rochester, Rochester, New York.

The present study sought to evaluate the incorporation of DL-leucine-1-C^{14} into Walker carcinoma 256 cell cultures and to determine the catabolic rate of the C^{14}-labeled cell proteins. The data demonstrate that C^{14} from DL-leucine-1-C^{14} is incorporated into cell proteins and into metabolic $C^{14}O_2$ at approximately the same rate. C^{14}-activity of the pre-labeled cell protein is transferred to the TCA-soluble fraction of the nutrient at an average rate of 12.5% per day. This catabolic rate is substantially the same as that obtained previously in experiments with DL-lysine-6-C^{14} labeling (Jordan, Miller and Peters, Can. Res., 19, 195, 1959). The loss of labeled cell protein to the TCA-precipitable fraction of the nutrient averaged 5.2% per day. The time for overall loss of one-half of the cell protein C^{14}-activity was approximately constant and equalled 2.7 days. In related studies, homogenates of Walker carcinoma 256 cells grown in spinner culture were proteolytically active as manifested by breakdown of hemoglobin and insulin-I^{131}. (Supported by a contract with the U. S. Atomic Energy Commission and by a grant from the Jane Coffin Childs Memorial Fund for Medical Research.)

4 *Biochemistry*

ENZYME PATTERNS AND FUNCTION OF BOVINE MAMMARY CELLS IN TISSUE CULTURE. K. E. Ebner,* C. R. Hoover,* Elizabeth C. Hageman,* and B. L. Larson. Div. of Biochem., Dept. of Dairy Sci., Univ. of Illinois, Urbana.

The specific activity of various enzymes (acid and alkaline phosphatases, lactoperoxidase, catalase, UDPGal-4-epimerase, glucose-6-phosphate dehydrogenase, isocitric dehydrogenase, lactic dehydrogenase, DPN-cytochrome-c-reductase and hexokinase) was examined at intervals in bovine mammary cells cultured from post-partum glands. Concomitantly, the incorporation of glucose-C-14 (uniformly labeled) into lactose was followed. In general, the specific activity of the enzymes decreased markedly by the end of the first week; thereafter the response was variable. A reversal occurred in the specific activities of the two phosphatases by the end of two weeks (acid became greater than alkaline), although the continuance of this pattern was not necessarily true in older cultures (one to two years in age). Lactoperoxidase was not found in the cells though this enzyme was present in milk. UDPGal-4-epimerase was initially present in the cells but activity disappeared between seven and ten days. Efforts to maintain the UDPGal-4-epimerase activity with hormones and tissue extract have been so far unsuccessful. Incorporation of glucose-C-14 into lactose continued up to two days after the cultures were initially established.

5 *Biochemistry*

A SERUMLESS MEDIUM FOR THE CONTINUOUS CULTIVATION OF CELLS OF NORMAL AND MALIGNANT ORIGIN. Alfred A. Tytell and Robert E. Neuman*. Virus and Tissue Culture Res. Div., Merck Institute for Therapeutic Research, West Point, Pa.

A serumless medium has been devised suitable for the growth and repeated subculture of Walker carcinosarcoma 256, sarcoma 180, KB and human heartcells in vitro. It consists of the customary amino acids, vitamins, minerals and glucose; together with insulin, salmine sulfate, pyruvate, D-galacturonate, methyl oleate, and lactalbumin hydrolysate. Each of the latter materials is shown to be required for maximal growth rates which are comparable to those on media containing dialyzed serum. The methyl esters of oleic, arachidonic, linolenic, linoleic acids as well as Tween 80 are stimulatory for cell growth. The methyl esters of caproic, capric, lauric, myristic, palmitic and stearic acids are inactive. Walker carcinosarcoma 256 cells grow in suspension (spinner flasks) in the serum-free medium. This is a further criterion for the adequacy of the medium. (This work was conducted under Contract No. SA-43-ph-1886 with the Cancer Chemotherapy National Service Center, Natl. Cancer Inst., Natl. Institutes of Health, U. S. Public Health Service.)

6 *Biochemistry*

EFFECTS OF 2-DEOXYGLUCOSE ON GROWTH AND METABOLISM OF HUMAN CELL CULTURES. Stanley Barban (intro. by E. C. Weinbach) Natl. Insts. of Health, Bethesda, Md.

It has been shown that 2-deoxyglucose inhibits glucose metabolism and hexose transport in bacteria, yeast and various mammalian tissues. In the present studies, the glucose analogue has been found markedly to inhibit the growth of cultured human cells. The degree of inhibition depended on the molar ratio of carbohydrate to inhibitor, and was related also to the carbohydrate substrate. At equimolar concentration of glucose or mannose and 2-deoxyglucose, there was almost complete inhibition of growth, which was reversible with added carbohydrate. Cells grown on fructose were more sensitive to the inhibitory effect.

Cell-free extracts of cultured mammalian cells prepared by sonic disruption phosphorylate 2-deoxyglucose to 2-deoxyglucose-6-phosphate. Unlike 2-deoxyglucose, the phosphorylated compound at 2×10^{-3}M inhibited by 50% the rates of the phosphohexoseisomerase and glucose-6-phosphate dehydrogenase reactions but had little or no effect on the hexokinase or 6-phosphogluconate dehydrogenase reactions. Results of studies on the metabolism of glucose-C^{14} by a human carcinoma cell culture grown in the presence and absence of 2-deoxyglucose will be presented.

7 *Biochemistry*

THE BIOSYNTHESIS OF CYST(E)INE IN MAMMALIAN CELL CULTURES. Vance I. Oyama*, Karl A. Piez, and Harry Eagle, Natl. Insts. of Health, Bethesda, Md.

The need for preformed cyst(e)ine in the growth of human and animal cells is often obscured by the fact that it may be present in the medium as a contaminating amino acid, bound to the serum protein and dissociated from it in culture. When suitably purified protein is used, cyst(e)ine becomes regularly essential for growth. Despite this requirement for exogenous cyst(e)ine, a number of human cell lines have been found to synthesize significant amounts. As shown by isotope dilution experiments, the S derives from the methionine of the medium, presumably by way of homocysteine. The C skeleton derives from glucose by way of serine. The specific activities of the protein cyst(e)ine and serine are consistently equal, whether C^{14} glucose or C^{14} serine is used as the precursor, and no matter what level of serine is added to the medium. Cells pre-starved in a cyst(e)ine-free medium failed to synthesize the amino acid unless homocystine or small amounts of cystine (I) were added to the medium.

8 *Biochemistry*

METABOLISM OF STEROIDS BY A STRAIN OF RABBIT FIBROBLASTS. M. J. Bryson* and H. E. Swim. Univ. of Utah, Salt Lake City and Western Reserve Univ., Cleveland.

Studies of the metabolism of steroids by strain U12 fibroblasts have been extended with RT6 cells (rabbit testicle) as a means of ascertaining whether the pattern of steroid metabolism might be used as a distinguishing characteristic of permanent lines of cells with respect to both species and tissue of origin. Cells on glass were incubated in growth medium supplemented with C^{14}-steroid. Steroid products were isolated from the combined cells and medium and were identified by a combination of chromatographic, chemical and isotopic techniques. Androstene-3,17-dione was converted to testosterone, dihydrotestosterone, androstane-3,17-dione, estrone and estradiol. Similarily, testosterone yielded a variety of related androgens, estrone and estradiol. Ten steroid products were isolated from progesterone but estrogens were not detected. Estradiol is converted to estrone and several other compounds which have not been characterized. Five products of cortisol metabolism have also been identified. The results indicate that the pattern of steroid metabolism by RT6 differs markedly from that of U12 particularily with respect to its ability to convert androgens to estrogens.

9 *Pharmacology*

CONTINUOUS GROWTH OF MYCOBACTERIUM LEPRAE MURIUM IN CULTURES OF MONONUCLEAR PHAGOCYTES. Y. T. Chang (intr. by S. M. Rosenthal) Leonard Wood Memorial, National Institutes of Health, Bethesda, Md.

Preliminary studies on the cultivation of M. leprae murium in mononuclear phagocytes obtained from peritoneal exudate of mice revealed that elongation of bacilli occurred in 49 of 50 cultures and that the number of bacilli increased 7-fold in 7 weeks although the cell population was reduced to about one-half (Fed. Proc. 18:373, 1959). It can now be reported that the number of bacilli continued to increase for an additional 3 weeks. In one experiment, macrophages which were infected with bacilli in vitro, were cultivated in a medium of 40% unfiltered horse serum, 48% Hanks' BSS, 2% beef embryo extract, and 10% spleen homogenate (spleen of mice, each in 5 ml BSS). Cells remained in good condition up to 73 days. A steady increase in the number of bacilli was observed throughout. At the 73rd day, the cell population was reduced to 40.4%; the average number of bacilli per cell had increased 15-fold; the total number of bacilli in the culture 6.5-fold; and the average length of bacilli 2.6-fold. Therefore, the bacillary mass had increased 17-fold (6.5 x 2.6) in a period of 73 days although the cell population was reduced to 40% of the original number.

10 *Pharmacology*

A SIMPLE CHEMOSTAT FOR THE GROWTH OF MAMMALIAN CELLS. Edward P. Cohen* and Harry Eagle. Natl. Insts. of Health, Bethesda, Md.

Cultured mammalian cells have been grown for indefinite periods in a chemostat modeled after that used for the controlled growth of bacteria. The apparatus consists basically of three glass reservoirs in series, containing (a) fresh medium, (b) a constant volume of cell culture, maintained in suspension by a magnetic stirrer bar, and (c) an overflow vessel. The middle culture vessel (b) is immersed in a constant temperature circulating water bath. The flow rate is controlled either by gravity, or by a peristaltic pump. The kinetics of growth in this system resembled those of bacteria under similar conditions. The rate of cell growth was a function of the rate of inflow of new medium. Under optimum conditions, the cells divided approximately every twelve hours. As the rate of input was slowed, the average generation time increased and the population density rose to a new plateau level. Experiments on the utilisation of various substrates as a function of flow rate will be described.

11 *Pathology*

INHIBITION OF CELL GROWTH IN VITRO BY ULTRAFILTRATES OF CHICK EMBRYO EXTRACT. Helen Maganini,* Milton Becker,* Richard Manassa* and George Hass. Dept. Path., Presbyterian-St. Luke's Hospital, Chicago, Illinois.

Aqueous chick embryo extracts were prepared. A portion was dialyzed against water for 3 weeks. A second portion was ultrafiltered. Part of the ultrafiltrate was dried while frozen and redissolved, 50 times concentrated, in the non-dialyzable fraction. Growth-promoting activities were assayed for 2 weeks in plasma clot cultures of chick liver. In plasma, supplemented with crude CEE, growth of cells was voluminous. Less growth occurred when the supplement was unconcentrated ultrafiltrate or the non-dialyzable fraction and still less when the supplement was Tyrode's solution. Concentrated ultrafiltrates with control of pH and ionic composition produced complete inhibition of growth, confirmed by microscopic study of serial sections of explants.

12 *Immunology*

DETECTION OF CELLS OF HETEROLOGOUS ORIGIN IN TISSUE CULTURE AND THEIR SEGREGATION BY THE USE OF DIFFERENTIAL MEDIA. Calvin M. Kunin,* Lyman R. Emmons* and William S. Jordan, Jr. University of Virginia School of Medicine, Charlottesville, Va.

A line of presumed pure human liver cells (Chang) received from a commercial source did not show expected virus sensitivity. Subculture into human serum medium resulted in emergence of virus sensitive cells which could be propagated in non-human serum medium without loss of sensitivity. The original culture was found to contain a mixture of 2 morphologically distinct cell types. These could be segregated by virtue of the lytic effect of unheated human serum upon one of them, and the destructive action of Coe or poliovirus on the other. Employing anti-HeLa cell rabbit serum and chromosome analysis, it was shown that the virus resistant line was of non-primate origin, probably mouse, while the virus sensitive line was of human origin. Predominance of one cell type over the other did not develop in horse serum medium following mixture of equal numbers of each line. Poliovirus CPE could not be demonstrated in such equally mixed cultures. Virus sensitive cells could be resegregated from mixed cultures by subculture into unheated human serum medium. A phenomenon which at first appeared to be transformation of virus resistant cells to virus sensitive cells thus was shown to be due to segregation of primate from a mixture of heterologous cells.

13 *Immunology*

STUDIES ON INFECTIOUS RNA FROM PURIFIED POLIOVIRUS AND COXSACKIE VIRUS. J. J. Holland*, L. C. McLaren, BH. Hoyer*, and J. T. Syverton, Dept. Bact., Univ. Minnesota, Minneapolis, and Rocky Mt. Laboratory, Hamilton, Montana.

Purified or crystallized enteroviruses yielded RNA with reproducible titers of 0.1% or more of source virus activity. Infectivity of extracted RNA was not reduced by chloroform extraction, hydrogen-bond disruption or disulfide-bond disruption. Association of infectivity with single-stranded polynucleotides was suggested by these findings and by kinetics of RNA inactivation by phosphodiester bond-breaking agents. Study of recovery of highly polymerized RNA by analytical ultracentrifugation, ultraviolet absorption and radiophosphorus labelling indicated that limitation of RNA infectivity involved entrance into the recipient cells rather than loss of RNA during preparative procedures. Comparative chromatographic behavior of RNA and whole virus indicated that infectivity was not associated with residual whole virus or virus protein. Factors influencing cell competence are being investigated. Infectious RNA obtained from cells during early phases of infection by whole virus resembles RNA from purified virus.

14 *Immunology*

DETECTION OF VIRAL DESTRUCTION OF CELLS IN CULTURES WITHOUT OVERT CYTOPATHOLOGY. D. R. Dubbs* and W. F. Scherer. U. Minn., Minneapolis, Minn.

Currently, detection of viruses in animal cell cultures usually depends on cytopathic effects observable under 100-200 x magnification. However, certain viruses are known to grow in cell cultures without overt cytopathology. In such instances, presence of virus is not manifested by ordinary microscopic examination, and special techniques are required to detect viral effects on cells. In studying Japanese encephalitis virus in L mouse fibroblast cultures (where virus multiplies without overt cytopathic effect) two simple methods for detecting cell destruction were developed: 1) measurement by cell counts of population differences between growing cells in infected and uninfected cultures and 2) observation of infected cells growing as sparse populations. When 1.5×10^5 or more L cells in a 16x125 mm. tube were infected with JE virus, cell growth was less in infected than in uninfected cultures. Retardation of cell growth in infected cultures depended on a balance between cell reproduction and cell destruction rather than on reproductive arrest of the entire cell population because with less than 10^5 cells, destruction was obvious in 2-7 days. Thus cell counts and infection of sparse cell populations served to detect viral cytopathic effects in virus-cell systems ordinarily manifesting no overt cytopathology.

15 *Immunology*

CONTINUOUS CULTIVATION OF ISOGENOUS CELL LINES FROM THE HUMAN EMBRYO. Eldon V. Davis and Vern S. Bolin (intr. by W. F. McLimans). U. S. Public Health Service, CDC, Phoenix, Arizona.

Attempts to develop sensitive tissue culture systems for isolation of possible viral agents responsible for epidemic gastroenteritis have led to the establishment of five serially transplantable cell lines from one human embryo. The organs of origin included brain, lung, heart, liver and intestine. An epithelial-like morphology was established after 3-5 serial transfers and has persisted for one year. Multinuclear giant cells are frequently observed with nuclei numbers ranging from 3-20. The spectrum of susceptibility to known enteroviruses varies with each cell line. Isolation of the cell line, the viral spectrum and morphological characteristics will be discussed.

16 *Immunology*

SELECTIVE TECHNIQUES IN THE CULTURE OF DIFFERENTIATED MAMMALIAN CELLS. Gordon Sato* and Stanley Wills* (intr. by L. Levine.) Grad. Dept. of Biochemistry, Brandeis Univ., Waltham, Mass.

Single cell platings of dispersed cell preparations from day old rat liver have given plating efficiencies of 10^{-3}-10^{-4}. The ornithine transcarbamylase (a liver specific enzyme) specific activity of two week old cultures is less than 5% of the inoculum. Antisera against these cultures which are absorbed with liver parenchyma are effective in inhibiting freshly obtained inocula. These findings indicate that the lack of differentiated properties in these cultures are not due to dedifferentiation but to selection of a cell type other than liver parenchyma in the inoculum. Selective techniques have been attempted to remove this hypothetical cell type. Cultures are initiated in the presence of antisera and in the absence of arginine. Although survivors of this treatment have been obtained, populations large enough to assess for liver properties have not.

17 *Immunology*

STUDIES ON THE NUCLEIC ACID METABOLISM OF RABBIT KIDNEY CELLS TREATED WITH 5-FLUOROURACIL AND INFECTED WITH PSEUDORABIES VIRUS. Albert S. Kaplan and Tamar Ben-Porat*. Div. of Microbiology, Dept. of Radiology, Albert Einstein Medical Center, Philadelphia 41, Pa.

Monolayers of rabbit kidney (RK) cells preincubated for 18 hours in the presence of 50 μg of 5-fluorouracil (FU) show a marked decrease in their ability to incorporate adenine-8-C^{14} into RNA and into DNA. This inhibition of DNA synthesis is not reversed by thymidine, by uridine, nor by a combination of the two. The incorporation of thymidine-2-C^{14} is arrested completely when the cultures are preincubated with FU. Infection of such cultures with pseudorabies (Pr) virus reverses this inhibition. An increased uptake of thymidine is observed after infection of RK cultures with Pr virus. Preincubation with FU does not prevent this increase due to infection. The reversal of the inhibition of thymidine uptake by FU after infection occurs at concentrations of FU as high as 1 mg/ml. Virus synthesis also remains unaffected by the presence of this drug. On the other hand, incorporation of adenine-8-C^{14} and uridine-2-C^{14} into RNA of cells exposed to FU is not significantly modified after infection. These results show that some disturbances in cellular function resulting from the action of this drug can be reversed by virus infection.

18 *Immunology*

NUCLEIC ACID AND PROTEIN SYNTHESIS IN ADENOVIRUS-INFECTED CELLS. Maurice Green and Geraldine E. Daesch (intr. by R. W. Schlesinger, Saint Louis Univ. School of Med., St. Louis, Mo.

KB cells growing exponentially in suspension were infected with 50 pfu (plaque forming units) per cell of type 2 adenovirus. After 1 hour, over 30% of the virus was adsorbed, fewer than 1% of the cells could be cloned, while over 70% were assayable as infectious centers. At 18 hours, each cell contained an average of 200 pfu. This increased to 2000 and 6000 pfu at 24 and 48 hours. During this period, cells in infected cultures increased maximally 1.2 to 1.3-fold, whereas uninfected cells increased 3 to 4-fold. Infected cells did not lyse, but appeared to increase in size. Dry weight analysis revealed a continuous increase in mass per infected cell which reached a weight of 2 to 2.5-times that of control cells at 36 hours after infection. Kinetic analysis of DNA, protein, and RNA revealed a continuous pileup of these polymers within infected cells (20-50% increase per cell at 12 hours, 40-70% at 24 hours, 60-100% at 36 hours after infection). Infected cells had an enhanced ability to incorporate radioactive phosphate. Incorporation was maximum about halfway through the latent period. Thereafter it decreased until at 36 hours it was less than that of uninfected cells. The relationship of increased polymer synthesis to viral development is being further studied.

19 *Immunology*

THE SUSTAINED GROWTH OF HUMAN AND ANIMAL CELLS IN A PROTEIN-FREE ENVIRONMENT. Harry Eagle. Natl. Insts. of Health, Bethesda, Md.

All suspension cultures so far tested (Hela, KB, 929) will grow, apparently indefinitely, in a completely defined and protein-free medium if the culture is equilibrated across a cellophane membrane with the same medium supplemented with as little as 2% dialyzed serum and pancreatic enzymes, the latter supplied as 0.25% "Vipkase." The pancreatic extract cannot be replaced by the non-essential amino acids. The culture compartment contains no chemically or immunologically demonstrable protein. The essential growth factor(s) dialyzing across the membrane are presumably small molecular weight compounds, either initially bound to the serum protein, or formed from it on proteolysis. The properties of the growth-stimulatory compound(s) will be discussed.

20 *Immunology*

MACROMOLECULAR GROWTH REQUIREMENTS OF SINGLE, DIPLOID, MAMMALIAN CELLS. H.W. Fisher,* R.G. Ham,* and T.T. Puck, Dept. of Biophysics, Univ. of Colorado Medical Center, Denver.

Development of methods for culture of diploid mammalian cells without change in chromosome constitution permits reliable comparison of growth requirements of these and hyperploid cells. The defined medium (purified fetuin and albumin, plus synthetic micromolecular constituents) which supports quantitative colony formation from single cells of the hyperploid S3 HeLa (78 chromosomes) fails to support growth of the diploid Chinese Hamster cell (22 chromosomes) or of diploid human cells (46 chromosomes). These 3 cell types have distinctive colonial morphologies and nutritional requirements. Like S3, the C. Hamster cell requires both fetuin and albumin and no other macromolecule for colonial growth on glass, though it has small molecular requirements not demonstrated by the hyperploid S3 (Ham, these Proceedings). While a defined medium is not yet available for the human diploid cell, it resembles S3 and the C. Hamster cell in glass-attachment and stretching response to fetuin. Each of these 3 cells exhibits distinctive characteristics in the fetuin-induced morphological change and its kinetics. Fetuin purified by fractional precipitation is partially inactivated on chromatography but the active fraction is unchanged in electrophoretic and sedimentation behavior, and in specific biological activity.

21 *Immunology*

SMALL MOLECULE REQUIREMENTS FOR CLONAL GROWTH OF SINGLE DIPLOID CHINESE HAMSTER CELLS. Richard G. Ham (intr. by T.T. Puck.) Dept. of Biophysics, Univ. of Colo. Med. Center, Denver.

Elsewhere in these Proceedings, evidence is presented that the macromolcular growth requirements for a clonal diploid cell line derived from the ovary of a Chinese Hamster, Cricetulus griseus, are identical with those of the S3 HeLa cell line (cf. abstract by Ham, Fisher and Puck). Colonial growth of isolated cells of the diploid hamster line cannot be accomplished in the "defined" S3 HeLa medium. However, when the medium is supplemented with thymidine, pyruvate, cysteine, iron, asparagine, and an increased amount of folic acid, colonial growth is accomplished. The first four supplements are essential for colonial growth. The latter two have proven beneficial in preliminary experiments, but do not appear to be absolute requirements. A purine source (hypoxanthine), present in the HeLa medium but apparently not required by HeLa cells, is also essential for the hamster cell line. The definite iron requirement of single hamster cells suggests an absolute dependence on oxidative respiration involving iron-containing cytochrome systems. The ability of HeLa cells to grow well in an iron deficient medium suggests a facultative anaerobic metabolism with little or no dependence on cytochrome-catalyzed oxidative respiration.

22 *Immunology*

THE CHANGING VIRAL SUSCEPTIBILITY OF A HUMAN CONJUNCTIVAL CELL IN CONTINUOUS CULTURE. Sam C. Wong (intr. by Edwin D. Kilbourne). Cornell University Medical College, New York, N.Y.

The Chang conjunctival cell line, like all other serially propagable cell lines, does not support full cycle multiplication of influenza viruses with production of infective virus. During studies of the production of incomplete non-infective virus in this cell line with N-WS and swine influenza viruses, but not with other strains, transmissible CPE (cytopathic effect) was sporadically observed. Continued serial passage of virus resulted in consistent CPE and the production of infective virus when such "adapted" swine or N-WS viruses were inoculated in high dilution (10^{-5}). Re-study of the original Chang line of conjunctival cells maintained in other laboratories has demonstrated that the original cell is still unable to produce infective virus - whether inoculated with pristine or cell-culture-adapted viruses. Study of original and derived cell lines strongly suggests that their differing viral susceptibility is on a genetic basis. Susceptibility is uninfluenced by conditions of cell cultivation. Although both the original cell line (which had been cloned) and derived cell line are now heterogeneous with respect to cell type, the latter are less heterogeneous than the parent cell line. The two lines on preliminary study show differences in chromosome numbers and morphology.

XUM

23 *Immunology*

COMPARISON OF CHROMOSOME NUMBER AND MORPHOLOGY IN TWENTY-EIGHT LINES OF MAMMALIAN CELLS. J. Johanna Clausen* and J. T. Syverton. Dept. Bact., University of Minnesota, Minneapolis.

Chromosome number and morphology were determined for twenty-eight established cell lines. Fourteen of these lines were "transformed"; fourteen were non-transformed. Chromosome morphology of primary cell cultures of each possible species of origin was determined for control purposes. In no instance was the chromosome morphology of the "transformed" cells similar to that of non-transformed lines or of primary cell cultures of the supposed species of origin. Five cell lines reportedly from rabbit, two from monkey, one each from cow and swine, were similar in chromosome morphology to human; one supposedly from monkey and four from human were similar to mouse. The comparative findings will be presented as photographs and by graphs.

24 *Immunology*

SEPARATION FROM HORSE SERUM OF GROWTH PROMOTING ACTIVITY FOR TISSUE CULTURE CELLS BY DIALYSIS. Don P. Metzgar Jr.* and Merwin Moskowitz. Dept. Biol. Sci., Purdue Univ., West Lafayette, Ind.

The Dialysate from serum dialyzed against synthetic medium or basic salt solution at 37°C has been found to be capable of substituting for whole serum in tissue culture growth media. The dialysate in combination with synthetic medium supports the growth of six cell strains thus far tested. The active factor(or factors) appears to be released from the serum as a function of time and temperature. Dialysis of serum was carried out at 4°C, 37°C and with serum which was allowed to stand at 37°C for 7 days or longer prior to dialysis at 37°C. The amount of growth promoting activity in the dialysate was greater when the serum was dialyzed at 37°C than when it was dialyzed at 4°C. The largest amount of growth promoting activity was obtained when the serum was allowed to stand at 37°C for a period of time before being dialyzed. A preparation was obtained which did not react with protein precipitants such as sulfosalicylic acid and which would support the growth of cells to the same extent as whole serum.

243. TOXICITY STUDIES AND DRUG METABOLISM

1 *Physiology*

COMPARATIVE TOXICITY OF THE ATLANTIC PUFFER FISHES OF THE GENERA SPHEROIDES, LACTOPHRYS, LAGOCEPHELUS AND CHILOMYCTERUS. Edward Larson, H. C. Lalone and Luis R. Rivas. Ichthyological Laboratory and Museum, Dept. Zool., Univ. Miami, Florida.

Spheroides maculatus was obtained from the Cocoa, Titusville and Miami areas. Spheroides testudineus also was obtained from Miami. The associated genera were taken from Biscayne Bay and consisted of Lactophrys tricornis, Lactophrys trigonus, Lagocephelus laevigatus and Chilomycterus schoepfi. Extracts of skin, muscle, liver and gonads of these were prepared and tested according to the method of Halstead. Skin, muscle, liver and gonads of Spheroides maculatus from Cocoa, Titusville and Miami areas have in most cases proven toxic with skin showing the highest degree of toxicity. In Spheroides testudineus the skin was always toxic and other organs exhibited a high degree of toxicity. Lactophrys tricornis and Lactophrys trigonus proved non-toxic; the liver of one Lagocephelus laevigatus was toxic, as was the liver of one Chilomycterus schoepfi. In the latter two genera skin, muscle and gonads were found to be non-toxic. In the anesthetized rat, skin extracts of Spheroides maculatus from Cocoa-Titusville have produced a decrease in blood pressure and have caused a depressor-pressor effect in the anesthetized opossum. Extracts of Lactophrys, Lagocephelus and Chilomycterus have elicited no essential change in blood pressure in the anesthetized rat or opossum.

2 *Physiology*

SOME PHYSIOPHARMACOLOGIC PROPERTIES OF CROTALUS VENOMS. Findlay E. Russell, Bryan A. Michaelis* and Roger Engelbert*. Lab. Neurol. Res., College of Medical Evangelists, Los Angeles, Calif.

Crotalus venoms are complex mixtures, chiefly proteins, many of which have enzymatic activity. The lethal and perhaps more deleterious effects of these venoms appear to be more closely associated with the non-enzymatic proteins than the enzymes and their combinations, though the enzymes certainly contribute to the over-all toxicity of the venom. The present study treats with certain cardiovascular, respiratory, central nervous system, and spinal fluid pressure changes produced in the intact cat by crude extracts of the venoms of C. atrox, C. horridus, C. viridis helleri, C. cerastes, C. ruber ruber and C. adamanteus. Lethal intravenous doses of these venoms produce a fall in systemic arterial, and pulmonary arterial pressures concomitant with an increase in venous and cisternal pressures, changes in respiration and the electrocardiogram and electroencephalogram. The significant relationships between these changes will be presented. The data indicate that while differences in the zootoxicologic effects of the venoms of these species and sub-species of rattlesnakes exist, they are primarily quantitative in nature. (Supported by grants B-1982 and H-3443 from the National Institutes of Health, P.H.S.)

3 *Physiology*

STEARIC ACID AS A POISON. G. E. Price* and R. H. Beutner College of Osteopathic Med. and Surg., Des Moines, Iowa

The toxicity of the higher fatty acids is believed to be low, but we found that they have a demonstrable toxicity when used in high doses. The method of Dr. Philip Harris of D.P.I. Rochester, N.Y. was used. The experiments were done on young white rats housed in individual cages at a temperature of 21°C. One half of the total food was stearic acid. The remainder was casein, glucose, salt mixture, cellulose and vitamin mixture. Water was supplied ad lib. The male rats died after an average of 8.2 days, the female rats after 10.2 days. Shortly before death the rats showed cardiac irregularities, demonstrable on the E.K.G. In some cases there was spasm and paralysis of the extremities. In other experiments the rats were given 3/10 the dose of stearic acid, in these cases death was very considerably delayed. The toxicity of stearic acid was markedly diminished by addition of glyceride oil, such as corn oil, even if as little as 5% was added.

This research was supported by grants from the National Heart Institute, P.H.S., Bethesda, Md. and from the American Heart Association of New York.

4 *Biochemistry*

FAILURE OF 2, 4-DINITRO-O-CYCLOHEXLYPHENOL AND ITS DICYCLOHEXYLAMINE SALT TO PRODUCE CATARACTS IN CHICKS AND DUCKLINGS. Bernard H. Armbrecht and Samuel H. Saver (introduced by Paul L. Day), Div. of Pharmacology, Food and Drug Admin., U. S. Dept. of Health, Education, and Welfare, Washington, D. C.

The study of H. C. Spencer et al., J. Ind. Hyg. Toxicol. 30, 10 (1948) with 2, 4-dinitro-o-cyclohexylphenol and its dicyclohexylamine salt in ducklings has been extended from 38 to 90 days. Chickens were used in a comparative study. The birds, 10 per level, were fed the compounds at 2500, 1000, 500, and 50 ppm with 20 controls in each species. No cataracts were observed in the survivors. Both species, when fed 2500 ppm of 2, 4-dinitrophenol, developed cataracts within 18 hours. One duckling, observed in a separate feeding, developed cataracts in 4 hours. Another, crop-tubed with 7-10 mg. suspended in water, developed cataracts in 3-1/2 hours, which disappeared within 3 days. No birds survived on the 2500 ppm level. Toxicity at other levels will be discussed.

5 *Biochemistry*

BIOCHEMICAL CHANGES IN GOSSYPOL TOXICITY. R. E. Davies*, V. S. Mohan* and J. R. Couch. Tex. Agri. Expt. Sta., College Station, Texas.

Dietary gossypol, gossypol acetate, gossypol-containing pigment glands from cottonseed meal, and gossypol acetate oxidized with peroxide depressed the growth of chicks. There was little evidence of anemia and no change in prothrombin time. Liver succinic dehydrogenase, cytochrome oxidase and xanthine dehydrogenase were decreased by gossypol, while endogenous respiration was unaffected. In vitro studies with livers from normal birds showed marked depression of succinic dehydrogenase, malic dehydrogenase and DPN - cytochrome C reductase following the addition of gossypol. Isocitric dehydrogenase, α-ketoglutaric dehydrogenase, xanthine dehydrogenase and cytochrome oxidase activities were slightly reduced by gossypol, while endogenous respiration was almost unaffected. Addition of ferrous or ferric ion (1.0 micromole per flask) reduced the inhibition of succinic dehydrogenase activity, and overcame the inhibition of DPN - cytochrome C reductase and cytochrome oxidase. Ferrous and ferric ions also overcame the inhibitory effect of ethanol on the latter two enzymes.

6 *Biochemistry*

STUDIES OF TOXICITY OF INTRAVENOUS FORMALDEHYDE. M. J. Orloff, G. Snyder, and C. Stevens (intr. by R.W. Virtue) Univ. of Colorado School of Medicine, Denver, Colorado.

Our studies of experimental ammonia intoxication have shown that intravenous formaldehyde has a marked capacity to bind blood ammonia. Since little is known about the effects of formaldehyde on living organisms, toxicity studies were undertaken. Investigation of acute mortality involved 240 dogs which were given formaldehyde intravenously in varying concentrations, rates of administration, time periods and total doses. The maximum non-lethal dose was found to be 309-450 mg/kg and the maximum non-toxic dose based on clinical observation, was found to be 288-424 mg/kg (0.7% solution given over 6 hours). Both of these dosage levels are greater than the dose which is effective in severe ammonia intoxication. Detailed acute toxicity studies involved 20 dogs which received non-toxic, moderately toxic and lethal doses of formaldehyde. The main effects of formaldehyde were a metabolic acidosis, which was severe at toxic dosage levels, and a consistent increase in the B globulin fraction of the serum proteins. There was no alteration of weight, hematopoiesis, liver function, PSP excretion, prothrombin time, blood sugar, BUN or serum Na, K, Cl, Ca, and P. Histologic studies of all tissues have failed, thus far, to reveal any abnormalities. In view of the absence of toxicity at effective dosage levels, continued investigation of formaldehyde is warranted. (Supported by USPHS Grant No. A-3048).

7 *Pharmacology*

EFFECT OF LONG-TERM CHLORTETRACYCLINE FEEDING ON FERTILITY OF RATS. D. W. Hallesy and C. H. Hine (intr. by Earl H. Dearborn) Experimental Therapeutics Research, Lederle Laboratories Division, American Cyanamid Company, Pearl River, New York.

A study to determine the effect of long-term administration of chlortetracycline on the fertility of rats was carried out. Food grade chlortetracycline was given in the diet at a 1% level. Control rats received the same diet without added chlortetracycline. Rats born to the first generation animals were weaned to the same diet as their parents. When these animals were of suitable age they were mated. The experiment was terminated when offspring from matings of second generation animals were of weaning age. It was found that addition of 1% chlortetracycline to the diet had no effect upon fertility of rats or the survival of their offspring when they were weaned to the chlortetracycline diet. A 1% chlortetracycline diet caused no significant change from control in body weight gains of rats from either the first or second generation when fed for approximately 35 weeks

8 *Pharmacology*

ORAL AND INHALATION TOXICITY OF DIPROPARGYL ETHER (DPE) AND 1,6 HEPTADIYNE (1,6 H). Frederick Sperling, Sperling Laboratories, Arlington 13, Virginia.

DPE oral LD_{50}'s in fasted male rats and rabbits and dogs of both sexes were 539, 168 and 913 mg/kg respectively. All species showed central depression and anorexia. Many rats developed pallor. Dogs vomitted after 3 hours. Autopsies showed lung congestion and hyperemia, cirrhotic livers, and blood non-coagulation. 1,6 H LD_{50}'s were 2.30, 2.62 and 3.83 gm/kg respectively. All species showed central depression. Anorexia was seen in rats and rabbits. Dogs showed good appetite after recovery from depression. Autopsies showed lung congestion and hyperemia in rats and dogs; infrequent lung lesions in rabbits. Rabbits and dogs showed lesions of the GI mucosa. Dogs had chicken-fat clots in the hearts. Air and N_2 generated vapors of both compounds were used for rat studies; air-generated only, for dog studies. Air-DPE LC_{50} for rats was 3.8 and for dogs 3.4 mg/L. N_2-DPE LC_{50} for rats was 2.6 mg/L. For rats, N_2-1,6 H LC_{50} was 5.5 and air-1,6 H was 2.5 mg/L. Dog LC_{50} was unobtainable. Reactions and pathology for each compound were as above, plus complete anorexia in all dogs. The central depression was segmental. Additionally, DPE-exposed dogs had chicken-fat clots at highest concentrations, and lesions of the GI mucosa at lower concentrations. Contract Nonr-2727(00). Office of Naval Research.

9 *Pharmacology*

TOXICITY OF HAFNYL CHLORIDE. T. J. Haley, N. Komesu* and K. Raymond. Dept. and Lab. of Nuc. Med. and Rad. Biology, Univ. of California at Los Angeles.

Intraperitoneal LD50 of hafnyl chloride is 112 (93.3-134.4) mg/kg. No body weight or hematological changes were produced when the chemical was fed at a level of 1% for 12 weeks. Topical application of HfCl4 on backs of rabbits gave a 24 hr. irritation index of 5 and penetrating ulcers developed on abraded skin. At doses of 0.5 to 2 mg/kg intravenously hafnyl chloride caused no observable pharmacological effects in the cat. A transient hypotension in both carotid and femoral blood pressure and a decrease in femoral blood flow occurred at 5 mg/kg. Complete cardiovascular collapse followed by respiratory arrest was seen after 10 mg/kg. ECG changes indicated a 2 to 1 or 4 to 1 heart block and cardiac arrest. Vascular responses to epinephrine, acetylcholine, histamine and vagal stimulation were not modified by hafnyl chloride. Ganglionic transmission and contraction of the nictitating membrane also were not modified. These effects were not prevented by atropine or reversed by epinephrine. pH also was not a factor and although the lethal dose was increased to 50-60 mg/kg by complexing with citrate the effects observed were related to citrate complexing calcium. The above results indicate that hafnium salts exert their greatest toxicity locally. Supported by USAEC Contract AT-(04-1)-GEN-12.

10 *Pharmacology*

TOXICITY STUDIES OF METHAMINODIAZEPOXIDE HYDROCHLORIDE. R.E.Bagdon (intr. by L.O.Randall) Dept. of Pharmacology, Hoffmann-La Roche, Inc., Nutley, N. J.

Methaminodiazepoxide (Librium(T.M.), 7-Chloro-2-methylamino-5-phenyl-3H-1,4-benzodiazepoxide hydrochloride) is a tranquilizing agent which is effective in a wide range of mental disorders. Administration of the drug to rats for 13 weeks at doses of 60 mg/kg, 30 mg/kg and 15 mg/kg was well tolerated; hematological, gross and microscopic findings were normal. Methaminodiazepoxide generally produced an increase in food consumption and, consequently, an enhancement in growth. Additional groups of rats were given methaminodiazepoxide for one year; the dosage schedule was 80 mg/kg, 40 mg/kg and 20 mg/kg; a first generation was also obtained. Methaminodiazepoxide did not adversely influence growth, food consumption, hemopoietic tissues or the process of reproduction; gross and microscopic examination of the tissues did not reveal pathological changes. Dogs received the drug in daily doses, ranging from 80 mg/kg to 5 mg/kg for 6 months; drug levels in plasma were 65.9 γ/ml to 7.1 γ/ml, respectively. The hematocrit, hemoglobin, total and differential leukocytes, bromsulfalein retention, serum bilirubin, alkaline phosphatase, thymol turbidity, SGO-T, SGP-T and serum lactic dehydrogenase activity remained within normal limits. These results indicate that methaminodiazepoxide exerts a low toxicity.

XUM

11 *Pharmacology*

TOXICOLOGY OF AMPHENIDONE (DORNWAL) A NEW PSYCHOPHARMACOLOGICAL AGENT. O.J. Plekss*, J.F. Reinhard, and J.V. Scudi. Maltbie Labs. Div., Wallace & Tiernan Inc., Belleville, N.J.

Because of the promise shown by 1-m-aminophenylpyridone-2 (amphenidone) in clinical trials, a report of its toxicology is presented here. Oral LD_{50}'s in mice and rats were 1300 and 2300 mg/kg, respectively. Lethal oral doses could not be established in higher species owing to emetic activity. The intravenous LD_{50} in the dog was in excess of 400 mg/kg. The pattern of toxicity indicated involvement of the CNS: ataxia, loss of equilibrium, and muscular flaccidity. Sleep was induced at lethal doses and then only in rodents. Subacute studies in the mouse (6 wks.) and chronic studies involving large daily doses in the rat (3 months) and dog (15 months) revealed no gross or microscopic evidence of toxicity referable to amphenidone. Hemograms, urinalyses, liver-function studies, and weight curves yielded results within normal limits. It is concluded that amphenidone is relatively non-toxic in the mouse, rat, cat and dog. These findings are consistent with the lack of side effects observed in clinical studies of more than 1,000 patients to date.

12 *Pharmacology*

EFFECT OF CONCENTRATION ON ORAL TOXICITY OF DRUGS. P. F. Moore*, G. K. W. Yim*, and T. S. Miya. Purdue University Dept. of Pharmacology, Lafayette, Indiana.

Borowitz's report [Fed. Proc. 16, 284 (1957)] that the oral toxicity of Na salicylate, Na pentobarbital and atropine SO_4 was enhanced by dilution of the dose was supported using procaine HCl. Rats dosed with twice the volume of water used in the dilute treatments did not show toxicity, thereby reducing the probability that water intoxication was the explanation. The onset of sleep obtained with a 0.5% solution of Na pentobarbital was significantly shorter than that with a 5% solution. A 0.8% solution of Na salicylate was absorbed better than a 25% solution and gave rise to higher plasma levels. The increased absorption was not due to increased intraintestinal pressure caused by large volumes. Mere difference in concentration was likewise not responsible for the increased absorption. The intestine absorbed markedly more salicylate than did the stomach. Dye studies showed that dilute solutions were more rapidly emptied from the stomach. The data indicates that the phenomena of increased toxicity and shortened onset of action are the result of this faster emptying which presents the material to a larger and better absorptive surface thus facilitating absorption to an extent beyond the ability of the organism to detoxify and/or excrete the drug.

13 *Pharmacology*

SUBACUTE ORAL TOXICITY OF TEN D&C COAL-TAR COLORS. Walter H. Hansen,* Diane C. Wilson* and O. Garth Fitzhugh. Division of Pharmacology, Food and Drug Administration, U. S. Dept. of Health, Education and Welfare, Washington, D. C.

As a continuation of previously reported studies (J. Pharmacol. 122, 29a, 1958) on the subacute oral toxicity of D&C colors, groups of ten weanling albino rats, equally divided as to sex, were fed diets containing D&C Red Nos. 8, 33, 34, 37, 39; Orange Nos. 3, 4; and Yellow Nos. 7, 10, and 11 at dosage levels of 0, 0.25, 0.5, 1.0 and 2.0 per cent for 90 days. All colors except Red Nos. 8, 37 and 39 were fed also at 5.0 per cent. Body weight and food intake were recorded weekly. Blood counts were done at 90 days. Organ weights were obtained at sacrifice. Yellow No. 7 caused kidney damage at the three highest levels. Red No. 37 and Yellow No. 11 caused liver enlargement at all levels. The spleens were enlarged on the animals being fed Red Nos. 8 and 33, and Orange Nos. 3 and 4. Minor effects were seen on Red No. 39. No gross effects were noted on Red. No. 34 and Yellow No. 10.

14 *Pharmacology*

DOSE-EFFECT RELATIONSHIP BETWEEN DRUGS AFFECTING THE SYMPATHETIC NERVOUS SYSTEM AND THE DEGREE OF HEPATIC LIPID INFILTRATION PRODUCED BY CARBON TETRACHLORIDE IN MICE. G. F. Kiplinger*, Alice Spencer*, and C. J. Kensler. Dept. of Pharmacology, Boston Univ., Boston, Mass.

It has been reported that the administration of ergotamine tartrate, dibenzyline, or reserpine to the rat treated with carbon tetrachloride will partially prevent the characteristic increase in total hepatic lipid (Calvert and Brody, Fed. Proc. 18:375, 1959). In the present study this observation has been confirmed in C3H mice; however, it has been found that there is a biphasic dose-response relationship between the increase in extractable lipid produced by CCl_4 and the dose of the blocking drug administered. Comparisons were made between normal control, CCl_4 treated, CCl_4 plus drug treated, and drug treated animals. Data will be presented indicating that, while low doses of ergotamine tartrate (3.0 mg/kg), dibenzyline (2-4 mg/kg) and reserpine (0.5 mg/kg) will partially prevent the CCl_4 effect, higher doses (6.0, 8.0, and 1.0 mg/kg, respectively) potentiate the CCl_4 effect. Ephedrine in all doses tested potentiates the CCl_4 effect. These results will be discussed in terms of the proposed role played by sympathetic innervation in the action of CCl_4 in producing fatty degeneration of the liver.

15 *Pharmacology*

FURTHER STUDIES ON MEPROBAMATE METABOLISM. Robert Wiser* and Joseph Seifter, Wyeth Inst. for Med. Res., Radnor, Pa.

Administration of carbonyl labeled meprobamate-C^{14} to rats, dogs or humans results in the urinary excretion of at least five labeled compounds as determined by paper chromatography in butanol-water (100:15 by volume). These compounds have Rf values of 0.8 (I), 0.6 (II), 0.4 (III), 0.1 (IV), and 0.05 (V). Compounds I and II have been previously identified as unchanged meprobamate and hydroxymeprobamate respectively (Walkenstein, et al, J. Pharmacol. & Exper. Therap. 123:254, 1958). Treatment of III with propionic anhydride gives a product with Rf = 0.9, indicating the probable hydroxylated nature of this substance. Metabolites IV and V both give positive periodate-pararosaniline tests for polyhydroxy compounds. Acid hydrolysis of an eluate of IV produces glucuronic acid and meprobamate-C^{14} (I), characterizing this as the N-glucuronide of the unchanged drug. Acid hydrolysis of V releases glucuronic acid and hydroxymeprobamate-C^{14} (II). This too appears to be an N-glucuronosyl derivative. Electrophoretic studies indicate that the carboxyl groups of both IV and V are free, making carbon atom 1 of glucuronic acid the probable point of conjugation.

16 *Pharmacology*

ACCELERATED DRUG METABOLISM IN RATS PRETREATED WITH DRUGS. A.H. Conney*, I.A. Michaelson* and J.J. Burns. Natl. Heart Inst., Bethesda, Md.

Pretreatment of rats for 4 days with phenobarbital, barbital, phenylbutazone, orphenadrine or aminopyrine causes several-fold increases in the ability of liver microsomes to metabolize hexobarbital, zoxazolamine, phenylbutazone and aminopyrine. The increased activity of the drug-metabolizing enzymes is paralleled in vivo by a shortened duration of action of hexobarbital, zoxazolamine, meprobamate, and carisoprodol. Pretreatment of rats with 3,4-benzpyrene stimulates the activity of the zoxazolamine-metabolizing enzyme in liver microsomes but not the hexobarbital-metabolizing system. In accord with this, 3,4-benzpyrene administration shortens the duration of zoxazolamine paralysis from 730 minutes to 17 minutes but does not shorten the duration of hexobarbital hypnosis. Pretreatment of rats with chlorcyclizine decreases the duration of action of hexobarbital and zoxazolamine. The finding that pretreatment of animals with one drug can speed up the metabolism of the same or another drug should be considered when evaluating the pharmacological activity of drugs that are given repeatedly, either alone or in combination.

17 **Pharmacology**

THE USE OF COMPUTERS FOR STATISTICALLY ANALYZING TOXICITY DATA. Otho Easterday, Peter Mumford* and Louis Potter*. Med.Res.Center and Applied Math. Div., Brookhaven Nat'l. Lab., Upton,N.Y.

The use of computers increases the speed and provides for a more elaborate convenient method for laborious statistical calculations. It is the purpose of this paper to present information regarding the use of a Remington Rand 409-2R for the probit transformation and maximum likelihood solution and of an IBM-610 for relative potency (RP) determinations of toxicity data (Finney, D.J., Probit Analysis (2nd Ed.),Cambridge,1952). The input data required for the probit transformation are: probit No., dose, No. tested, response,nat.mortality, degrees of freedom (df), log dose, emperical probit, and selected dose for calculation. The output in addition to the input is: expected probits, fiducial limits (FL) (dose,probit), regression quantities ($\bar{x}$, b, $\bar{y}$), variance and standard error (SE) of b, g, heterogeneity factor, t, Chi^2, log dose sign, Sxx, Syy, Sxy and SWO. The RP input data in addition to that cited above are: probit No's., df (total, parallelism, residual heterogeneity), t, Chi^2 (parallelism, residual heterogeneity), variance ratio, constants (1, 5, 2.3, 1.96). The RP output for each drug is: intercept, slope, log LD50, V(b'), g, RP 10M, SE, FL, relative dose M ± FL, ± FL M, and statistical significance. The time required for each probit is about 12 min. and for one RP is about 5 min. (Supported by the U.S.Atomic Energy Commission)

18 **Pharmacology**

A SENSITIVE ASSAY FOR THALLIUM IN URINE UTILIZING EXTRACTION WITH 2-OCTANONE. William B. Stavinoha*, Joe B. Nash and George A. Emerson. Dept. of Pharmacology, Univ. of Texas Medical Branch, Galveston, Texas.

A 25 ml. aliquot of a 24 hr. urine sample is mixed with 10 ml. of nitric acid and evaporated to dryness. Ten ml. of water, 1 ml. of hydrobromic acid and 0.05 ml. of bromine are added. The sample is heated to remove the bromine. Extraction of the thallic ion is accomplished by shaking the solution for one minute with 10 ml. of 2-octanone. The phases are allowed to separate, and the organic phase is washed with 10 ml. 1M hydrobromic acid and aspirated into the Beckman DU spectrophotometer equipped with photomultiplier and flame attachment and estimated in an oxy-acetylene flame at 377.6 mμ. Experiments carried out with the aid of radioisotopic Tl^{204} show that the procedure results in recovery of 99.7% of the thallium in the sample of urine. Results of thallium estimations when 5, 10 and 20 mcg. of thallium respectively are added to urine are: 5±0 mcg., 10±0.5 mcg. and 20±0.6 mcg.

19 **Pharmacology**

NICOTINE ABSORPTION FROM THE URINARY BLADDER OF THE DOG. Joseph F. Borzelleca (intr. by Paul S. Larson.) Medical College of Virginia, Richmond, Va.

It has been reported that 10% of a given dose of nicotine is excreted unchanged in the urine of dogs. To determine the role of bladder absorption on nicotine toxicity, the following studies were conducted in adult, male, mongrel dogs anesthetized with pentobarbital sodium, 30 mg/kg, I.V. Blood pressure, respiration and electrocardiograms were recorded. In some animals, the ureters were exposed, cut and doubly cannulated to collect urine and to perfuse fluids into the bladder. An indwelling urethral catheter was used in all dogs to either instill solutions into the bladder or as an outflow during perfusion. (-)-Nicotine in 0.9% NaCl, or in appropriate buffer, was used at a temperature of 37°C. When doses of 10, 25, 50 mg/kg of (-)-nicotine were placed into the bladder, changes in blood pressure, respiration and EKG were noted usually in 2 minutes. When the solutions were perfused through the bladder, the onset of nicotinic effects seemed to be more rapid with the more alkaline solutions. Macht (J. Urol. 2:221, 1918) reported that nicotine is very slightly absorbed from the canine bladder if at all. However, our studies in dogs support the findings of Travell (PSEBM 45:552, 1946) who showed that nicotine is absorbed from the cat bladder and this absorption is pH dependent. (Supported by NIH Grant, RG-7064.)

20 **Pharmacology**

HEMATOLOGIC ALTERATIONS PRODUCED IN THE DOG BY THE ADMINISTRATION OF 2-CHLORO-10-(3-DIMETHYLAMINOPROPYLIDENE)-THIAXANTHINE. Harold M. Peck and Samuel E. McKinney,* Merck Institute for Therapeutic Research, West Point, Penna.

2-Chloro-10-(3-dimethylaminopropylidene)-thiaxanthine was given orally to dogs in daily doses of 5, 10 or 20 mg./kg. as a part of a toxicologic evaluation of the agent prior to clinical trial for efficacy in the therapy of mental diseases. After a few weeks of drug administration, toxicity was manifested by leukopenia (as low as 1.1 thousand/ml.), a marked shift of the Schilling index to the left and increased sedimentation rates. When drug administration was stopped, the hemograms returned to normal within two to four weeks. Reinitiation of drug administration resulted in a return to the abnormal hematology. Two closely related thiaxanthine derivatives were also studied. One of these, 2-chloro-10-(3-diethylaminopropylidene)-thiaxanthine, produced leukopenia and was hepatotoxic. The second agent, 2-chloro-10-(3-dimethylaminopropyl)-thiaxanthine, was cardiotoxic for the dog, but did not cause changes in the hemogram. These studies again demonstrate that relatively simple alterations of chemical structure can result in varied types of toxicity.

21 **Pathology**

SHORT TERM EXPERIMENTS WITH CHLORPROMAZINE IN RATS: 1. HISTOLOGIC STUDIES. Leon Roizin, M. A. Kaufman* & Bruno Miles*. N. Y. S. Psychiatric Institute, N.Y.

Rats injected with toxic doses of chlorpromazine (10-50 mg./100 g.b.wt.) and sacrificed after 4-24 hrs. showed increased sudanophilia of hepatic cells in some lobules and of some renal tubule cells. Occasional glial reaction and neuronophagia were found in cerebral cortex, lenticular nucleus, thalamus and hypothalamus. Various stages of chromatolysis were seen throughout the CNS. Chief histologic findings in rats given 0.6 mg./100 g.b.wt. did not markedly differ from those of control rats except for many large cells resembling megakaryocytes and lipid-laden cells in the spleens of treated animals, the latter also in one control animal. Chronic bronchopneumonia was common in all groups. Livers of some rats sacrificed 16-18 hrs. after 10 mg./100 g.b.wt. and of another group sacrificed 9 wks. after 1.2 mg./100 g.b.wt. showed some inflammatory foci. Thus, short term experiments with chlorpromazine in rats showed histologic changes in hepatic cells, renal tubules, spleen and CNS mainly after toxic doses. (Supported by P. H. S. Grant 1665-C2)

244. TUMORS

1 **Physiology**

TRANSPLANTABILITY CHANGES OF A MOUSE TUMOR AFTER PASSAGE THROUGH TOLERANT HOMOLOGOUS RECIPIENTS. Carlos Martinez, Harvey Kelman * and Robert A. Good. (Depts. of Physiology and Pediatrics, Univ. of Minn. Medical School, Minneapolis 14, Minn.)

Incidence of "takes" and progressive growth of a mouse mammary carcinoma of the A strain when transplanted into F_2 hybrids produced by crossing (A x Z) F_1 X (A x Z) F_1 mice was determined after this tumor was passed through the following recipient mice: (A x Z) F_1, Z tolerant of A tissue and A tolerant of Z tissue. Controls consisted in F_2 mice transplanted with the original A tumor. Tolerant animals were produced by intravenous injection at birth of homologous spleen cells. Results were: 1) Incidence of tumor takes in F_2 recipients after one passage in (A x Z) F_1 was 23% of 48 animals; 2) After passage in Z tolerant of A tissue, incidence in F_2 was 27% of 55 mice; 3) After passage in A tolerant of Z, incidence in F_2 was 8% of 49 mice; 4) Direct transfer of original tumor to F_2 mice resulted in an incidence of 2% of 44 animals. These findings will be discussed in the light of adaptive histocompatibility changes occurring in mouse tumors after residence in homologous recipients similar to those described by Barret and Deringer after passage through susceptible F_1 hybrid individuals.

2 *Physiology*

ABNORMAL REPAIR AND METASTASIS IN EXPERIMENTAL GASTRIC CANCER. Rhoda Grant. Univ. of Ill. Med. Coll., Chicago.

An omental adenocarcinoma originating below a gastric lesion was found in a rat (1 of 6 from a separate study) 180 days after implanting 9,10-dimethyl-1,2-benzanthracene in the gastric wall. Serial sections showed a direct spread from omentum to spleen and liver, but no continuous growth from lesion to tumor, such as characterized the carcinomas previously produced by this method, and no primary tumor in any other organ. Regeneration defects found before in gastric tissues at carcinogen sites were present, i.e. replacement of chief and parietal cells by mucous cells, reversal in growth direction of the glands, absence of muscularis mucosae and basement membrane, defective submucosal tissues. Dilated precapillary lymphatics marked by Periodic Acid Schiff positive material in the lumens and in abnormal mitotic and undividing cells which they contained extended from the base of the lesion to the tumor and were an unusual feature. It was concluded that the tumor was metastatic from the lesion. Hypothetically mucin from the mucous cells in the lesion or a PAS positive product from cells of other tissues damaged by the carcinogen, in finding an abnormal outlet, sets up a malignant change in cells which are not normally exposed to it. (Supported by USPHS Grant No. C-3086).

3 *Pharmacology*

A STRAIN OF NEUROSPORA CRASSA REQUIRING 2-ACETYLAMINOFLUORENE: GENETICS AND ALTERNATIVE GROWTH REQUIREMENTS. Philip S. Thayer, Jean F. Ames, Nicholas diTullio, and William Wallace Biology Laboratory, Arthur D. Little, Inc., Cambridge. (Intr. by C. J. Kensler, Dept. of Pharmacology, Boston Univ., Boston.)

The riboflavin-requiring temperature mutant of Neurospora (strain 51602) can become tolerant to certain previously inhibitory azo dyes and to 2-acetylaminofluorene (AAF; N-2-fluorenylacetamide) (Thayer, Mahoney, and Kensler; Salzberg). Genetic segregation from crosses of the adapted culture with a wild-type yielded a strain with an apparent growth requirement for an azo dye or AAF, and a reversed temperature sensitivity. Experiments on the specificity of this requirement have shown that it is not met by riboflavin, other B vitamins or hydrolyzed yeast nucleic acid. Growth is supported, in the absence of carcinogen, by amino acids (hydrolyzed casein, or certain single amino acids) or by certain organic solvents (dioxane and related compounds). The nature and concentration of the inorganic nitrogen source has a significant effect on the level of AAF required for maximum growth. Genetic analysis has shown that the alternative requirements for AAF and amino acids (but not dioxane) and the effects of inorganic nitrogen remain associated through two further generations of crosses. Dissection of asci has given results consistent with, though not proving, a single gene segregation.

4 *Pharmacology*

STUDIES ON THE MECHANISMS OF ACTION OF N-METHYLFORMAMIDE. Joseph A. DiPaolo and Richard G. Rosenfield (intr. by C. Ambrus). Roswell Park Memorial Institute, Buffalo, New York.

This study purposed to elucidate the mode of action of N, methylformamide (NMF) which preferentially inhibited acriflavin induced respiratory deficient yeast mutants but not the wild type of S. cerevisiae 1741 (Bristol Lab.), S. carlsbergensis ATTC 4226, S. cerevisiae var. ellipsoideus and Yeast Foam. Neither type was inhibited with urethan, formamide, N,N-Dimethylformamide or N-methylacetamide pointing to specificity of NMF. When grown anaerobically NMF failed to inhibit the growth of the wild type cells thereby ruling out a direct glycolytic effect. Comparison of mutant growth on a minimal medium with and without NMF showed that NMF exerted a static rather than fungicidal effect on the mutants. Compounds of 10^{-2}M from RNA-DNA metabolic pathways were used in a liquid medium containing $(NH_4)_2SO_4$, KH_2PO_4, necessary vitamins, minerals and 4% sucrose. Growth was measured turbidimetrically at 525 mu. after 66 hrs. incubation at 29°C. Adenine showed the greatest reversing action using 0.085 M NMF with S. carlsbergensis mutant III M10. Other compounds tested showed varying degrees of reversal; 2,6-diaminopurine (DAP) failed to reverse NMF inhibition in yeasts although Skipper et al. (Can. Res. 15:143, 1955) demonstrated reversal in E. coli. DAP prevented the reversing action of adenine. (Supported by a grant CY 3553 from NIH.)

5 *Pathology*

EXPERIMENTAL PATHOGENECITY OF SCOPULARIOPSIS BREVICAULIS V. HOMINIS IN CFW MICE. Z. T. MANKOWSKI (intr. by S. P. Reimann) Institute for Cancer Research, Philadelphia.

Pathological changes could be obtained by injecting Scopulariopsis brevicaulis in saline or 5% mucin in CFW mice. Shortly after an injection of a mucin suspension the organisms could be found in the spleen and could sometimes be demonstrated there even after one year. The predominant pathological change is the formation of a granuloma. Advanced amyloidosis of the spleen, liver and kidneys was observed. Often nuclear inclusion bodies were seen in the liver. Mice which were treated with cortisone showed a virus-like hepatitis within two weeks (also with Russel's bodies visible). In chronic infections hyperplasia of the lymph nodes was seen, often with a distortion of their architecture. In addition three lymphoblastomas were observed. Among 42 newborn mice at the age 2-4 days at the time of injection either with saline or mucin suspension, 5 hepatomas, 1 reticulum cell sarcoma, 1 lymph node hyperplasia bordering on neoplasia and 1 severe atrophy of the pancreas were observed.

6 *Pathology*

CONDITIONS DETERMINING INFECTION OF MIGRATING SKIN EPITHELIUM OF RABBITS WITH SHOPE PAPILLOMA VIRUS. Charles Breedis. School of Med., Univ. of Pa., Phila. 4, Pa.

Shope papilloma virus is usually inoculated by methods that involve trauma, thus making study of mechanisms of infection difficult. In the experiments to be reported, virus suspensions were placed directly in contact with thin sheets of epithelium that had been migrating from small autografts of skin within large transparent chambers. Concentrated suspensions of virus increased the normal migration rate 10-fold. Progressive dilutions of virus yielded intermediate rates of migration after progressively increased incubation periods. Such slowly migrating papilloma epithelium could not be successfully superinfected by concentrated suspensions applied to it one or two weeks later. Heat-inactivated virus apparently enhanced infection instead of interfering with it. Some of these results can be explained by the development of immunity to virus during the experiment. It is also possible that: 1) Papillomas consist of a mixture of normal and infected cells; or 2) Different degrees of infection of a cell may occur. (Supported by a grant from the National Institutes of Health, USPHS.)

7 *Immunology*

IMMUNIZATION AGAINST EHRLICH ASCITES CARCINOMA WITH NITROGEN MUSTARD TREATED CELLS. David M. Donaldson and James A. North*, Department of Bacteriology, Brigham Young University, Provo, Utah.

Mice can be protected against a transplant of Ehrlich ascites carcinoma by immunization with tumor cells inactivated in vitro with x-irradiation (Proc. Soc. Exp. Biol. & Med. 101:204, 1959). Since nitrogen mustard, mechlorethamine hydrochloride (HN_2) has many radiominetic properties, immunization experiments were carried out with cells exposed to HN_2 in vitro. Tumors could not be successfully transplanted following a 30 min. exposure of fifty million cells to doses of HN_2 greater than 0.01 mg, but treatment with these HN_2 inactivated cells resulted in enhanced immunity against subsequent transplants. As the concentration of HN_2 was increased above 0.01 mg the protection due to immunization decreased. Multiple injections of the HN_2 treated cells reduced the mortality from 100% to less than 7%. No protection was demonstrated when immunization followed tumor transplantation. (Supported by Grant C-4608 N. I. H., P. H. S).

245. TUMORS—Ascites

8 *Immunology*

FIBROSARCOMAS IN HAMSTERS AT SITE OF INJECTION FOLLOWING INTRACEREBRAL OR SUBCUTANEOUS ADMINISTRATION OF S.E. POLYOMA VIRUS, Flaviu C. A. Romanul*, Bernard Roizman and Charles N. Luttrell*, The Johns Hopkins Medical Institutions, Baltimore, Md.

Single or multiple tumors developed intracranially, and in the lungs, liver, heart and kidneys in 67, 58, 54, 49 and 13%, respectively, of 63 hamsters inoculated intracerebrally at birth with $10^{4.5}$ mouse embryo T.C. ID_{50} of cell-free partially purified polyoma virus. The same dose induced tumors at site of injection, and in the liver, lungs, heart and kidneys in 48, 43, 39, 30 and 13%, respectively, of 23 hamsters injected into subcutaneous tissue; intracranial tumors were absent. Hemorrhagic lesions in liver and lungs contained invasive tumor cells. Heart and kidney tumors were occasionally hemorrhagic and frequently encapsulated. Subcutaneous tumors were encapsulated and occasionally hemorrhagic. Intracranial tumors appeared to originate from the meninges and were non-hemorrhagic. Most of them invaded brain substance growing around blood vessels. A few tumors in the cerebellopontine angle were encapsulated and were manifest by disorders of equilibrium. All tumors were fibrosarcomas, containing collagen and reticulum. (Supported by grants from USPHS, C-4204 and E-1230).

9 *Immunology*

TUMOR FORMATION, ANTIBODY RESPONSE, AND THE RECOVERY OF S. E. POLYOMA VIRUS FROM TUMORS IN THE SYRIAN HAMSTER. Bernard Roizman and Philip R. Roane, Jr.* From the Department of Microbiology, The Johns Hopkins University, Baltimore, Md.

Titration of partially purified cell-free virus in newborn hamsters revealed that hemagglutination-inhibiting or complement fixing antibody reached peak levels between 30 to 60 days and was a more sensitive indicator of virus than tumor response. Hamsters with tumors at one site, particularly the site of injection, occasionally lacked demonstrable antibody. Virus was demonstrated in 2 of 14 tumor homogenates by injection into mice and in 7 of 22 tumors by injection of fluids or cells from tumor cell cultures into newborn hamsters. Most isolations were from homogenates or cultures of tumors from animals with high antibody titers. Hamsters injected with live cells from tumor cultures frequently developed invasive tumors at site of injection but no antibody. It is concluded that (1) lack of demonstrable antibody in some of the hamsters with tumors is due to virus multiplication that is inadequate to stimulate a good antibody response, (2) virus isolation is more probable from animals in which virus multiplied extensively and which also developed high antibody response, and (3) tumor formation may not be related to the extent of virus multiplication in the hamster. (Supported by a grant from the USPHS #C-4204).

10 *Immunology*

EFFECT OF TOTAL BODY X-IRRADIATION ON THE VIABILITY OF METASTATIC TUMOR CELLS. Horace Goldie, Gadson J. Tarleton,* Lonnie Deadrick,* and Odis Strong* (Depts. of Oncology and Radiology, Meharry Med. Coll., Nashville, Tennessee).

CFW mice were inoculated i.p. with 10^7 S-37 cells and after two days, irradiated with 200r (Group I), 300r (II), 400r (III) and 600r (IV). Group V: untreated controls. Two days later all mice developed about the same amount of ascitic growth; 0.2 ml of heart blood of each mouse (donor) was injected i.p. into a new mouse (recipient)—see Goldie, & al: Detection of Metastatic Cells by i.p. Grafts—Cancer Res. 13:566 (1953). Next week ascites tumors were found in 85 to 100% of recipients of blood from I, II, III and V, but only in 10% of recipients from IV. In analogous experiments with smaller inocula (10^6 or 10^5 cells) 70 to 95% of negative results were recorded in Groups III and IV and 10 to 20% in I, II, and V. Conclusion: Doses of irradiation insufficient to affect significantly primary i.p. growth (Tarleton & Goldie, 1954) inhibited the viability of metastatic tumor cells in a high proportion of mice; this effect was directly proportionate to the dose of irradiation and inversely to the primary tumor inoculum. It is presumed that fractionated x-irradiation may reduce the number of metastatic tumor cells in the blood of patients, but it will be sought to replace total body irradiation by local irradiation. (Grants NIH & AEC)

1 *Physiology*

A CRABTREE-LIKE EFFECT IN HOMOGENATES OF ASCITES CELLS. R. Cereijo-Santalo (intr. by R. L. Post) Physiology, Vanderbilt Univ., Nashville, Tenn.

The endogenous respiration of ascites tumor cell homogenate (prepared in an omnimixer) is inhibited by D-glucose, D-mannose, fructose and DL-glyceraldehyde but not by 3-methylglucose, 2-deoxyglucose, glucose-6-phosphate or fructose-1, 6-diphosphate. The inhibition produced by glucose varies from 10 to 45%. Addition of yeast hexokinase usually increases the Crabtree effect especially in the less sensitive preparations. The endogenous respiration is very sensitive to changes in pH. The drop in pH produced by the addition of glucose could account for the decrease in respiration. However, 1) both 4 mM glucose and 4 mM mannose drop the pH from 7.2 to 6.4, but at a concentration of 4 mM, glucose inhibits respiration and mannose does not. 2) The addition of 10 mM lactic acid drops the pH to 6.4 and causes no inhibition. 3) Glucose-6-phosphate and fructose-1, 6-diphosphate partially release the inhibition produced by glucose although the pH still drops to 6.3.

2 *Biochemistry*

INCORPORATION OF NUCLEIC ACID AND PROTEIN PRECURSORS INTO ASCITES TUMOR CELLS. E.R.M. KAY. Department of Biochemistry, University of Rochester School of Medicine and Dentistry, Rochester.

A study has been made of the incorporation of C^{14} formate, C^{14} DL Alanine, and C^{14} Glutamine into the nucleic acids and proteins of nuclear and cytoplasmic fractions of Ehrlich-Lettre ascites tumor cells both in vivo and in vitro experiments. Time course experiments showed labelling of cytoplasmic proteins to be considerably greater than for nuclear proteins. With formate, activity is located essentially in the serine moiety as seen in radioautographs of the protein hydrolysates. Nuclear RNA, DNA and cytoplasmic RNA were hydrolyzed with perchloric acid to the constituent bases which were separated by paper chromatography. C^{14} formate was incorporated into their adenine, guanine and thymine. According to the specific activity of the bases, nuclear RNA is synthesized much more rapidly than cytoplasmic RNA. Evidence is presented for the possible nuclear origin of some of the cytoplasmic RNA, in accord with earlier suggestions (Biochem. J. 62: 160, 1956). The effects of some anti-tumor agents have been studied. Among these, the antibiotic Sarkomycin has been found to interfere with protein synthesis, and evidence is presented for interference with de novo purine synthesis through an involvement with glutamine metabolism.

3 *Biochemistry*

THE UPTAKE OF COLLOIDAL Au^{198} BY EHRLICH ASCITES CARCINOMA CELLS IN VITRO. Donald F. Hoelzl Wallach and Donna Ullrey (intr. by M. J. Hunter) Harvard Medical School, Boston, Mass.

Washed ascites cells exposed to colloidal gold particles 3-7 mμ in size in vitro, aerobically and without added substrate, take up particles at a slow, continuous rate for several hours. Addition of glucose produces a 40% increase in aerobic gold uptake. Fluoride and iodoacetate are strongly inhibitory; 10^{-4} M dinitrophenol (DNP), itself without effect on gold absorption, produces a 50-100 fold increase in gold uptake in the presence of glucose. In cells preincubated with DNP, this effect appears 15-20 minutes after addition of glucose and continues for at least 1 hour. Gold uptake without glucose is 3-5x higher anaerobically than in air, but in the presence of glucose, anaerobic gold uptake is as great as that observed aerobically with DNP. Under these conditions, a 10% cell suspension is able to remove more than 10% of the gold in the medium per hour. The stimulation of gold uptake by DNP or anaerobiosis is completely abolished by fluoride and iodoacetate. It is believed that gold particles enter by pinocytosis and that this process is activated during glycolysis. (Supported by a grant from the National Institutes of Health.)

4 *Pathology*

CONTINUITY AND RATE OF DNA SYNTHESIS IN ASCITES TUMOR CELLS. J. L. Edwards*, and Arthur L. Koch* (intr. by Philip McMaster.) Depts. of Pathol. and Biochem., College of Med., Univ. of Florida, Gainesville, Florida.

In vivo studies of Ehrlich ascites tumor cells during the first five days of growth in peritoneal cavities of mice consisted of the following: (1) determination of growth curves by direct enumeration of cells; (2) estimation of the duration of each phase of the mitotic cycle based on incidence of cells in different phases; (3) radioautographic studies to determine the proportion of cells in different phases of the mitotic cycle that incorporate tritiated thymidine during a single brief exposure to this precursor of DNA; (4) estimation of the rate of incorporation of tritiated thymidine at different times during the period of DNA synthesis by comparison of mean grain counts over nuclei in radioautographs at different times following exposure to tritiated thymidine.

The assumptions underlying these experiments and observations concerning the duration of the period of DNA synthesis and its relation to the mitotic cycle are discussed. It is concluded that DNA synthesis is continuous, occupying a period of 8.5 hours during the interphase and that the average rate of synthesis is approximately constant. (PHS Grants E-1566 and E-1542 made this study possible.)

5 *Immunology*

A COMPLEMENT-DEPENDENT SYSTEM OF NORMAL SERUM LETHAL TO MOUSE TUMOR CELLS. M.Landy, J.G.Michael*, R.-J.Trapani*, B.Achinstein*, M.W.Woods* and M.J.Shear*. Natl. Cancer Inst., Bethesda, Md.

Study of the system in normal serum lethal for Gram-negative bacteria led to a search for humoral systems cidal for tumor cells. Human serum, interacted in vitro with Sarcoma 37 ascites, was found lethal for these cells. Sera from 9 other species were similarly cytotoxic, whereas sera from 7 others were not. The lethal effect was manifested by: failure of the tumor cells to proliferate in an appropriate host; altered permeability to eosin; morphologic changes; and, as shown by Woods et al.(Fed.Proc.,1960), cessation of glycolytic activity. Serum cytotoxicity was lost either upon absorption at 4°C. with S37, which removed a specific factor, or upon heating for ½ hour at 52°C., which inactivated C'2. Divalent cation and the recognized components of complement were required for the lethal effect. The factor absorbed by S37, which behaved like natural antibody, could also be removed by: Ehrlich and Krebs carcinomas; Hepatomas 119 and 134; Sarcoma 180; and by cell suspensions from solid tumors (S37; Melanoma S91). Cell suspensions of some normal mouse tissues (liver, lung, kidney, heart, muscle, erythrocytes) did not remove this factor, but spleen cells absorbed it completely.

246. TUMORS–Chemotherapy

1 *Biochemistry*

BIOCHEMICAL ACTIVITY OF 4-AMINOPYRAZOLO(3,4-d)PYRIMIDINE IN THE EHRLICH CARCINOMA. Alan C. Sartorelli*, Barbara A. Booth*, and Thomas A. McCoy. Biomedical Div., Samuel Roberts Noble Foundation, Inc., Ardmore, Oklahoma.

Pyrazolopyrimidines whose basic dissociation constants approximate that of adenine showed carcinostatic activity. Also, 4-aminopyrazolo(3,4-d)pyrimidine (APP) toxicity can be reversed by adenine, other purines, their ribosides, or ribotides in several biological systems. Therefore, effects of APP on purine metabolism and on protein synthesis have been investigated in Ehrlich ascites cells. In vivo administration of 30 mg/kg APP prior to adenine-8-C^{14} did not alter the rate of adenine incorporation into polynucleotide adenine or guanine. Injection of APP one hour before glycine-2-C^{14} resulted in approximately 90% inhibition of glycine utilization for polynucleotide adenine or guanine, with about 50% inhibition present 6 hours after drug. The rate of glycine-2-C^{14} incorporation into residual protein was retarded by APP. Maximum inhibition of 50% occurred 6 hours after drug. To determine whether APP interfered with protein synthesis or altered metabolic disposition of glycine, the effect of APP on lysine-U-C^{14} incorporation into protein was determined. The results paralleled those obtained with glycine. These studies demonstrated that APP retarded both de novo purine synthesis and protein synthesis in Ehrlich ascites cells.

2 *Biochemistry*

INHIBITION OF EHRLICH MOUSE ASCITES TUMOR GROWTH BY CORDYCEPIN. Nicholas M. Kredich, Donald V. Jagger, and Armand J. Guarino (intr. by A. A. Christman). Dept. of Biol. Chem., The Univ. of Michigan, Ann Arbor.

Cordycepin, a nucleoside containing adenine and a branched 3-deoxypentose, has been isolated and crystallized from liquid cultures of Cordyceps militaris. In this study purified cordycepin has been injected as a possible antitumor agent into mice bearing the Ehrlich Ascites Tumor. All injections of tumor, cordycepin, and saline were intraperitoneal. Tumor bearing control animals received equivalent volumes of 0.9% NaCl, and in every instance drug or saline was administered daily over a 7-day period. In 3 series of experiments the following were determined: The ability of the host to tolerate varying doses of cordycepin; the level of compound necessary to produce an inhibitory effect on the tumor; and the ability of cordycepin to inhibit advanced tumor growth. It was found mice could tolerate between 150-200 mg of cordycepin per kg of body weight. Inhibition of tumor growth, in many instances complete, was noted at dosages ranging from 15-200 mg per kg of body weight when the drug was started on the day of tumor innoculation. When the initial administration of drug was begun on animals in which the tumor was 5 days old a dosage of 150 mg per kg of body weight resulted in a significantly increased survival time.

3 *Biochemistry*

THE COMPARATIVE ANTI-TUMOR ACTIVITY OF THE DIAZOMYCINS. J.J.Oleson, A.R.Reith*, R.S.Thie*, K.J.Mjos*, and L.A. Calderella*. Chas. Pfizer & Co., Inc., Maywood, New Jersey.

Three new anti-tumor antibiotics, Diazomycins A, B, and C, have been isolated from Streptomyces ambofaciens. These diazo-type antibiotics possess high anti-tumor activity against several experimental tumors. Three mouse tumors, Sarcoma 180, Carcinoma 755 and Leukemia L-1210 are all markedly inhibited by these antibiotics. The optimum doses for Diazomycins A, B, and C are approximately 0.4, 0.1 and 0.4 mg/kg/day against all three tumor systems. Some differences that appear in the pattern of activity of these antibiotics against the three tumors will be discussed. The compounds are also active against well established tumors in all three of the above systems. The human tumor HS#1 is not inhibited by daily doses of Diazomycin A. Various dosage schedules and routes of administration were studied.

4 *Biochemistry*

THE INTERACTION OF CHLOROQUINE MUSTARD WITH ASCITES TUMOR CELLS R.J.Rutman, W.J.Steele* and C.C.Price,* Harrison Laboratory of Chemistry, Univ. of Pennsylvania, Phila.

Numerous investigations have demonstrated that variations in the R group of nitrogen mustards ($RN(CH_2CH_2Cl)_2$) produce alterations in toxicity and effectiveness. We have shown that such variations also produce different patterns of reaction with DNA and are now investigating the invivo interactions with tumor cells. By exposing ascites tumor cells to varying concentrations of tritiated chloroquine mustard (CQM·H^3), the relative uptake by cell constituents can be related to cytotoxic action. In this manner, we have found that the uptake by phospholipid and acid soluble fractions is less than 5% of the total uptake, while proteins account for ~50% of the bound isotope, and ½ of this is alkali labile. The reaction with cell protein commences at concentrations as low as 5×10^{-7} M CQM, in advance of significant cytotoxic effects. The specific activity of both protein and RNA increases rapidly with increasing [CQM·H^3]. In contrast, experimentally significant reaction with cellular DNA requires higher CQM concentrations, corresponding to >99% cytotoxicity, and the uptake does not increase as rapidly as it does for the proteins. However, even the lowest significant levels of H^3 uptake by DNA correspond to $>10^5$ molecules of CQM incorporated per cell. (This work was supported by a USPHS Grant.)

5 *Pharmacology*

EFFECT OF MECHLORETHAMINE ON TUMOR GLYCOLYSIS AND DIPHOSPHOPYRIDINE NUCLEOTIDE CONTENT. Ian M. Fraser (intr. by C. M. Gruber), Coll. Med. Evangelists, Loma Linda, California.

Mechlorethamine (nitrogen mustard, HN2) has been found to inhibit the aerobic and anaerobic glycolysis of Ehrlich ascites and several other mouse tumors in vitro. The degree of inhibition depended upon the concentration and the time of exposure; 10^{-5} M HN2 ultimately produced marked inhibition after 3-4 hours. The glycolysis of tumor homogenates fortified with diphosphopyridine nucleotide (DPN) and other cofactors was much less sensitive to HN2. Fluorometric analysis for both DPN and DPNH in Ehrlich ascites tumor cells revealed that a marked fall in the content of these substances accompanied the inhibition of glycolysis by HN2. Nicotinamide, a DPNase inhibitor, at a concentration of 10^{-2} M protected against the inhibition of glycolysis and the fall in DPN content produced by HN2. It is concluded that the fall in DPN content is probably the cause of the inhibition of glycolysis but further investigation is required to determine the reason for this fall. (Supported by a grant from the National Cancer Institute, #CY-4482.)

6 *Pharmacology*

CARCINOSTATIC EFFECTS OF NITROMIN. T. DasGupta and A. Lane (intr. by Jean Sicé) Chicago Med. School, Chicago, Ill.

Recent reports (Ishidate, Brock) have described the carcinotoxic properties of large doses of methylbis(2-chloroethyl)amine N-oxyde. The transplanted tumors failed to grow or regressed; the animals recovered and were considered cured. The present investigation established the carcinostatic effects of repeated small doses of Nitromin on recent subcutaneous implants of the Walker carcinosarcoma. The malignant growth was delayed for at least two weeks, but did resume after the cessation of the therapy. The latent period of the tumor was appreciably and consistently prolonged beyond the seven days which followed the end of the treatment. The survival of the rats, from the time of the tumor appearance, remained the same as that of the control animals. The incidence of metastases was definitely decreased in the treated animals; no secondary pulmonary growth was then observed and the number of lymphatic metastases was considerably smaller than in the untreated animals. The carcinostatic effect of Nitromin cannot be attributed to a metabolic deficiency of the animals; none of the rats lost weight until the tumor was actively growing. (Grant CY-2646, US. PHS.).

7 *Pharmacology*

STUDIES ON INTRAVENOUS PLASMIN IN HUMANS. William L. Wilson and Francis Naso (intr. by Lewis C. Mills) Hahnemann Med. College and Hospital. Philadelphia, Pennsylvania.

It has previously been demonstrated that intravenous plasmin in humans will produce fibrinolysis. Two streptokinase activated plasmin preparations have been compared at different dosage levels noting the side reactions and effect on the euglobulin lysis times. The difference in toxicity and effect on the euglobulin lysis time when compared on a unit to unit basis appears to be significant. In an attempt to develop a regime of maximum dosage over a prolonged period of time, patients have been given varying doses of plasmin at 6 hour intervals. There appears a cumulative effect as noted by shortening of the euglobulin lysis times with successive doses and in some cases marked fluctuation in the fibrinogen levels. Several patients had bleeding phenomenon while receiving plasmin including intracranial hemorrhage. The circumstances and outcome regarding the complications will be discussed. Serial euglobulin lysis times and fibrinogen levels on the above cases will be shown .

8 *Pharmacology*

E-73 - AN ANTI-TUMOR AGENT. W. S. Marsh*, A. L. Garretson*, and A. Farrell*. (Intr. by Jean Scholler). John L. Smith Memorial for Cancer Research, Chas. Pfizer & Co., Inc., Maywood, N.J.

A new streptomyces albulus species designated in this laboratory as BA 4105 produces the anti-tumor agent E-73. A description of this strain of streptomyces albulus, its culture characteristics and fermentation studies will be discussed. The antibiotic is inhibitory to saccharomyces cervisiae and can be assayed by an agar plate disc method. A good yield of the compound can be produced in a fermentation medium containing cerelose, soy bean meal and salts. E-73 Base and several of its derivatives, Dihydro E-73, E-73 Acetate and Desacetyl E-73, were tested against a number of animal tumor systems. The comparative activity of these four compounds will be presented. The most sensitive tumors were found to be the human tumor HS#1, Carcinoma 755 and Sarcoma 180.

9 *Pharmacology*

THE INFLUENCE OF DISULPHONYL MANNITOL ON NEOPLASTIC TISSUES IN MAN. H.R. Bierman, G.J. Marshall* and G.M. Timmis.* Institute for Cancer and Blood Research and School of Medicine, College of Medical Evangelists, Los Angeles, and Chester Beatty Research Institute, London, England.

The ratio of tumor inhibition to toxicity with some antineoplastic agents is related to solubility. The water soluble hexamethylene, 1:6-Dimethane sulphonyl-D-mannitol (DSM) combines relatively low toxicity with powerful inhibition of the Walker and 'August' rat carcinomas. In vivo assays in 5 patients revealed decrease in ascitic tumor cell count within 24 hours accompanied by profound chromosomal and cytoplasmic aberrations following the intracavitary instilla- of DSM. Subsequently, 15 patients with advanced neoplastic diseases received DSM by various routes in amounts ranging from 1.0 to 100 grams. Definite anti-tumor effects were recorded in melanoma, metastatic adenocarcinoma of the colon, breast and kidney. DSM in moderate doses caused generalized weakness, dryness of the skin, hematodepression and alopecia. Recovery from hematodepression was generally prompt following cessation of administration. Despite objective tumor regression, generalized prolonged improvement in these patients is yet to be demonstrated.

10 *Pharmacology*

THE CARCINOSTATIC EFFECT OF CERTAIN PSYCHOPHARMACOLOGICAL AGENTS. BENEDICT O'MALLEY, ROBERT WILLHEIM AND PAUL FLUSS. (INTR. BY JOHN V. SCUDI.) FAIRLEIGH DICKINSON UNIV. AND INST. OF APPL. BIOL. NEW YORK.

CERTAIN PSYCHOPHARMACOLOGICAL AGENTS INHIBIT CELL GROWTH, WHICH IS REVERSED BY ADENOSINE-5-MONOPHOSPHATE (O'MALLEY'58). STUDIES WERE UNDERTAKEN TO DETERMINE THE EFFECTIVENESS OF PHENOTHIAZINE DERIVATIVES AND AZACYCLONAL ON NEOPLASTIC CELL GROWTH. TREATMENT OF ASCITES TUMORS IN MICE WITH THE PHENOTHIAZINE DERIVATIVE PROCHLORPERAZINE RESULTED NOT ONLY IN REGRESSION OF TUMOR MASS IN VIVO, BUT COMPLETE ABSENCE OF CANCER CELLULAR ELEMENTS AT AUTOPSY IN A NUMBER OF ANIMALS. PROCHLORPERAZINE INCREASED MARKEDLY THE CARCINOLYTIC POWER OF HUMAN SERUM AND EFFECTED A CHROMATIN CLUMPING IN SALINE SUSPENSIONS OF CANCER CELLS. MICROSCOPICALLY THERE APPEARED TO BE A BREAKDOWN IN CYTOPLASMIC AND CHROMOSOMAL NUCLEIC ACID MATERIAL. THE PHENOMENON OF CARCINOLYSIS WAS INHIBITED BY ADENOSINE-5-MONOPHOSPHATE AND OTHER NUCLEOTIDES. SPONTANEOUS TUMORS IN THE DOG AND CAT REGRESSED WITH THE THERAPEUTIC USE OF PROCHLORPERAZINE. IT IS OF IMPORTANCE TO DETERMINE THE ACTION OF THESE COMPOUNDS ON CANCER CELLS AS POSSIBLE CHEMOTHERAPEUTIC AGENTS IN CANCER.

XUM

11 *Pathology*

CHEMOTHERAPY OF CANINE CANCER WITH DIHYDRO E-73. John R. McCoy* (intr. by R. B. Stebbins) Bureau of Biological Research, Rutgers University, New Brunswick, N.J.

A total of 25 cases of spontaneous canine cancer were treated with this actidione derivative, including 14 dogs afflicted with malignant lymphoma, 5 cases of mammary adenocarcinoma, 3 cases of mast-cell sarcoma, 2 cases of epidermoid carcinoma, and 1 case of thyroid adenocarcinoma. Regressions and significant remission periods were noted in the majority of the malignant lymphoma cases. A variable response was obtained in the cases of mast-cell sarcoma, epidermoid carcinoma and thyroid adenocarcinoma. There was no measurable therapeutic effect in any of the mammary adenocarcinoma cases. There was no detectable toxic effect on the circulating blood cells and there was no depletion of plasma protein in dogs receiving the antibiotic intermittently for a period up to one year.

247. TUMORS–Growth

1 *Physiology*

EXERCISE, FATIGUE AND TUMOR GROWTH. S. Hoffman*), K.E. Paschkis and A. Cantarow. Jefferson Medical College, Philadelphia, Pa.

There is but scant information on the effects of vigorous exercise on tumor growth. In the experiments reported in the literature the effects were considered and discussed from the angle of effects of "stress" on tumor growth. Reports on the effects of extracts of fatigued muscles on growth of tetrahymena suggest a more specific effect of muscular exercise. In our experiments the growth of Walker 256 in rats vigorously exercised was compared with that of controls who were individually housed in small cages permitting them only a minimum of movements. In all instances the weight of the tumors of the controls exceeded considerably and significantly that of exercised rats; in several instances there was complete tumor regression in the exercised animals; no difference in carcass weight was observed between the two groups. Subsequently excised rat muscles were suspended in saline solution and electrically stimulated to "fatigue" evidenced by cessation of contraction upon stimulation.

Injection into tumor bearing rats, of a preparation made of the bathing fluid of a fatigued muscle markedly inhibited tumor growth, whereas a similar preparation from non-fatigued muscle was ineffective.

2 *Pathology*

PATHOLOGIC CHARACTERISTICS OF THE MELANOTIC AND AMELANOTIC MELANOMA OF HAMSTERS. J.C.Rosenberg*, P. Lober*, and B. Zimmermann. Univ. Minn. Hosp., Mpls., Minn.

The following data was obtained from a study of the Fortner amelanotic #3 and melanotic #1 melanomas. Subcutaneous and intramuscular implantation of 5 mm. fragments of melanotic melanoma produced the demise of the animal by 66.3 days (S.D.±29.6); the amelanotic by 38.7 days (S.D.±19.2). Gross metastases were present in 95% of the hamsters with the amelanotic tumor and 89% of those with the pigmented neoplasm. Those from the melanotic melanoma were found in the lungs of 72% of the animals; in 17% there was spread to the lymph nodes. Gross metastases from the amelanotic tumor were never found in the lungs but were present in the lymph nodes of all of the hamsters dying with metastases. Liver and kidney metastases were found in 52% of the animals with the amelanotic tumor but only 6% of those with the melanotic variety. The cells of the pigmented neoplasms did not appear as undifferentiated or as pleomorphic as those of the tumors which did not produce melanin. The amelanotic melanoma's propensity to metastasize via the lymphatics resulted in a diffuse form of liver involvement due to the presence of tumor cells in the portal areas. (Supported by a grant from the Minnesota Division of the American Cancer Society).

3 *Pathology*

CHANGES IN MORPHOGENETIC POTENTIAL OF TERATOCARCINOGENIC EMBRYOID BODIES IN VIVO AFTER PROLONGED MAINTENANCE IN VITRO. G. B. Pierce, Jr., F. J. Dixon, Jr. and E. L. Verney*. Dept. of Pathology, Univ. of Pittsburgh, Pittsburgh 13, Pa.

Teratocarcinogenic embryoid bodies produced in the ascitic fluid of strain 129 mice by an intraperitoneal injection of minced teratocarcinoma (LS 402 VI A) were washed and minced in Ca^{++} and Mg^{++} free BSS. Some were grafted subcutaneously in strain 129 mice to produce control tumors while others were explanted in plasma clots in roller tubes. Cultures were fed media containing fetal calf sera and Hanks's BSS 20:80 in Eagle's medium. After 6 months in vitro, explants were grafted subcutaneously in mice while others were serially sectioned for microscopic analysis of the cell types grafted. These explants were composed of cores of embryonal carcinoma and neuroepithelium invested by yolk sac or squamous cells. Whereas brain predominated in control teratocarcinomas, in the majority of those developed from cultured explants muscle predominated at the expense of brain. Striated muscle comprised 80% of the volume of some tumors (step section technique). The incidence of muscle producing teratocarcinomas decreased with serial transplantation but persisted in some lines for as many as 4 generations. These data suggest that differentiation of embryonal carcinoma, the stem cell tissue of teratocarcinomas, may be amenable to direction by experimental means.

4 *Pathology*

GROWTH OF HUMAN CANCER CELLS IN NEWBORN RATS. Leon J. Kutner and Chester M. Southam (intr. by L.D.Hamilton) Sloan-Kettering Institute for Cancer Research, New York City.

Intravenous injection of 2 to 3 million tissue-cultured HEp2 cells (human epidermoid carcinoma of larynx) into newborn rats resulted in progressive growth of the heterotransplanted cells with stunting and death of the rats in 3 to 5 weeks. Mortality among 84 rats injected with HEp2 when less than 24 hours old was 92% (immediate deaths excluded) while 37 litter-mate controls injected with balanced salt solution had only 2¾% mortality by age 5 weeks. Gross tumor masses of the injected cell type almost completely replaced the lungs of nearly all of the HEp2-injected mice, and smaller masses were occasionally found in heart, brain, adrenal, and kidney. Rats inoculated at age 3 days showed no stunting, mortality, or gross lesions.

5 *Immunology*

RELATION OF POLYOMA VIRUS TO TUMOR IN VIVO. Karl Habel. National Institutes of Health, Bethesda, Md.

Fibrosarcomas produced by inoculation of newborn mice and hamsters with polyoma virus can be transplanted in adults of the corresponding species. Although virus could be demonstrated in the original tumor, no further evidence of its presence could be found after the first transplant in the hamster. On the other hand, in a mouse tumor transplant series virus could be found associated with the tumor for the first 5 transplants but not thereafter. Virus-negative tumor transplants in tissue culture were irradiated with X-ray and UV-light, but virus production could not be induced. When virus was given to newborn hamsters along with antiviral serum at a different site of inoculation, tumors appeared after a longer incubation period and some of these tumors apparently were not producing virus since the animals were negative for virus antibodies. These findings suggest that although virus is necessary for inducing normal cells to become malignant, the virus is not necessary for maintenance of the tumor.

248. TUMORS–Metabolism

Physiology

TRANSAMINATION IN TRANSPLANTED AND SPONTANEOUS TUMORS. Michael A. Chirigos*, Stewart R. Humphreys*, and Abraham Goldin. Laboratory of Chemical Pharmacology, National Cancer Institute, Bethesda, Md.

Extracts of normal mouse liver and of transplanted and spontaneous tumors were investigated for the presence of the amino acid-parahydroxyphenylpyruvate transaminase reaction. Activity was measured as micromoles of tyrosine formed per ml. of enzyme preparation. Seven L-amino acids were studied as possible amino donors. In normal liver, marked activity was observed with phenylalanine, glutamic acid, glutamine, alanine and methionine, while aspartic acid and cysteine showed lesser activity. Similar results were obtained with Hepatoma 129. Carcinoma C-63 exhibited high activity with glutamic acid as an amino donor, and Sarcoma 180 with phenylalanine. Glioma 26, Lymphosarcoma 4946B and Carcinoma 755 had low activities. The spontaneous tumors tested were also relatively inert. The observed differences in transaminase activity serve to further characterize normal and aberrant tissues.

Physiology

A CALORIMETER FOR THE CONTINUOUS RECORDING OF THE ENERGY METABOLISM OF SMALL LABORATORY ANIMALS. William C. White (intro. by E. R. Buskirk.) Nat. Cancer Inst., Nat. Insts. of Health, Bethesda, Md.

Instrumentation is described that measures and records the energy expenditure and oxygen consumption of the experimental animal on continuous test periods up to 72 hours. Heat production, respiratory water elimination, oxygen consumption and carbon dioxide production are sampled at two-minute intervals throughout the experimental period. The calorimeter is designed on the adiabatic principle, with sensitive thermistor transducers used to maintain an isothermal condition and to measure the heat removed from the cage. The large volume of data accumulated in an animal experiment makes the use of electronic data-processing equipment not only economically feasible but almost a necessity. The use of an analog-to-digital converter permits recording of the measurements on punched paper tape which is converted to punched cards and processed on the IBM-650 computer. The calorimeter is being used to compare the total energy metabolism in the normal rat with the animal growing a transplanted tumor. The calorimeter described has proved to have an overall efficiency within 1.5%.

Biochemistry

CHANGES IN HEXOKINASE AND DEHYDROGENASES FOR GLUCOSE -6-PHOSPHATE AND 6-PHOSPHOGLUCONATE DURING CARCINOGENESIS IN THE HAMSTER CHEEK POUCH. D.B.McNair Scott, A.L.Morris*, A.B.Reiskin* and A.M.Pakoskey*. University of Pennsylvania, Philadelphia, Pa.

A 0.5% mineral oil solution of 9-10-dimethyl-1,2-benzanthracene was painted tri-weekly onto the cheek pouches of hamsters. Gross tumors appear in all animals within 7 weeks. Pouches were excised at intervals during the first week and weekly for 13 weeks. Enzyme activities were determined on extracts of whole pouches or tumors and tissue from which tumors had been removed. After minor fluctuations of all three enzymes during the inflammatory reaction and quiescent period, hexokinase (Hex.) and phosphogluconate dehydrogenase (PGD) fell to 50% and 40% of control after 2 weeks, slowly rising toward control values during the period of hyperplasia and tumor formation. In tumors the Hex. was 1.5 to 5.5 times, and PGD 2 to 7 times the control. In the residual pouch tissue both enzyme levels were normal to 3.5 times. The glucose-6-phosphate dehydrogenase increased continuously from 4 or 5 weeks, and activities were 2 to 5 times the control in both tumors and surrounding tissue.

Biochemistry

CHEMICAL PROPERTIES OF THE TUMOR-DAMAGING COMPLEX SEPARATED FROM S. MARCESCENS. E. Merler, A. Perrault and P. T. Mora (intr. by M. J. Shear.) Natl.Cancer Inst., Bethesda, Md.

A new preparation of the endotoxic agent separated from Serratia marcescens exhibited biological effects similar to those of previous samples. The complex migrated in an electrophoretic field as an homogeneous component at pH 6,7 and 9. On sedimentation (in 1% NaCl) two peaks were observed having uncorrected sedimentation constants 3.74 and 14.2 x 10^{-13}. Titration of an isoionic solution indicated 75 titratable COOH and 25 NH_2 per 100,000 M.W. unit. The complex contained 1.2% N. and 0.3% organically bound P. After acid hydrolysis chromatographic separation of the water soluble fraction indicated: glucose, glucosamine, possibly muramic acid, and an unknown component with $R_{glu.}$ 0.4, in the carbohydrate fraction; small amounts of glutamic acid and aspartic acid in the minor amino acid fraction. The complex was not degraded by a variety of glucose-splitting enzymes. Treatment with hot 1% HAc separated from the polysaccharide a lipide fraction (10%), chiefly (9.8%) $CHCl_3$ and acetone soluble, the remainder $CHCl_3$ sol., and acetone insol. None of these exhibited tumor necrotizing effects.

Biochemistry

ENZYME LEVELS IN THE SARCOMA-180 OF MICE TREATED WITH 6-MERCAPTOPURINE. P.J. Fodor and D.A. Clarke,* Division of Enzymology and Metabolism and Division of Experimental Chemotherapy, Sloan-Kettering Institute for Cancer Research, New York, N.Y.

Swiss albino mice were implanted with sarcoma-180 and received i.p. injections of 6-mercaptopurine (MP) for the next 7 successive days; the daily dose was 75 mg/kg body weight, divided into two parts given at 7-8 hour intervals. MP was suspended in saline containing 0.5% of 6-carboxy-methylcellulose (CMC). The control group received injections of CMC in saline. The tumors were excised on the 8th and 9th day after implantation. Catheptic activity (substrate:hemoglobin), ATPase and AMPase (substrate:adenosine-5-phosphoric acid) were significantly increased ($p < 0.01$) in homogenates prepared from tumors of MP-treated hosts as compared to those of the control group. The soluble nitrogen/ml. in tumor homogenates from MP-treated hosts was significantly decreased ($p < 0.001$). CMC-saline injections cause a marked rise in tumor cathepsin, but fail to affect ATPase, AMPase or the amount of soluble nitrogen. Injections of saline have no effect on any of the above. Additional enzymes are under study and further data will be presented.

Biochemistry

THE EFFECT OF AZO-DYE CARCINOGENESIS UPON 5-HYDROXYTRYPTAMINE (SEROTONIN) DEAMINASE ACTIVITY OF RAT LIVER. D. E. Kizer and S. K. Chan (intr. by S. H. Wender.) The Samuel Roberts Noble Foundation, Inc., Ardmore, Oklahoma.

Previous reports indicated hepatomas were deficient in enzymes associated with the conversion of tryptophan to kynurenine. A second minor catabolic pathway involving 5-hydroxytryptophan has been demonstrated in mammalian tissues. Since rat liver possesses the enzyme 5-hydroxytryptamine deaminase, which catalyzes the oxidative deamination of serotonin to 5-hydroxyindoleacetic acid, our purpose was to determine (a) whether transplanted hepatomas retained this enzyme and (b) whether deaminase activity was altered during azo-dye carcinogenesis. In all experiments ammonia production was the criterion for deamination of serotonin. Following establishment of valid assay conditions for deaminase activity in perfused rat livers, the activity in normal rat liver was compared to that in transplanted hepatomas. The activity in rat liver was 5- to 6-fold greater than the activity in transplanted hepatomas. When animals were fed a semi-synthetic diet containing 3'-methyl-4-dimethylaminoazobenzene no appreciable change in deaminase activity was observed throughout 90 days. These observations indicated that although the enzyme was virtually absent in transplanted hepatomas the precancerous trauma exerted no appreciable influence upon serotonin deaminase activity of liver.

XUM

7 *Biochemistry*

METABOLITES OF CARCINOGENIC AROMATIC AMINES AND AZO DYES AS METAL ION COMPLEXING AGENTS. J. H. Weisburger, P. H. Grantham*, and E. K. Weisburger*. Natl. Insts. of Health, Bethesda, 14, Md.

Chemical carcinogens of the aromatic amine type, such as N-2-fluorenylacetamide, are extensively metabolized by various species to give the corresponding aminohydroxy derivatives [cf. Adv. Cancer Research 5, 331 (1958)]. Studies dealing with the metal ion chelating properties of such derivatives were performed. Potentiometric titration methods were used to evaluate the formation constants. The complexes were not very soluble in many of the ordinary solvents tried, but good results were achieved in 70% aqueous dimethylsulfoxide. In addition, several of the metal chelates were isolated. Satisfactory elemental analyses corresponding to 2:1 complexes were obtained with o-aminophenols, and 1:1 complexes with 5-dimethylamino-2-phenylazophenol. The complexing ability of 2-fluorenamine itself, and that of the 7-hydroxy derivatives was very weak or nil. However, 2-amino-1- and 3-fluorenol, 3-amino-2-fluorenol, and 2-amino-4-phenylphenol were moderately strong chelating agents. Ranges of the first and second stability constants of the complexes with Cu^{++} were 11-12 and 9-10, resp., with Ni^{++} 7.5-8.6 and 4.8-6, with Co^{++} 7.0-7.3 and 5.5-6.0, with Zn^{++} 7.5-8.0 and 5.5-6.4, with Pb^{++} about 7 and 6. Thus, metabolic transformation of aromatic amines and azo dyes leads to compounds with metal ion chelating properties.

8 *Biochemistry*

MITOCHONDRIAL TRIOSEPHOSPHATE ISOMERASE. Carl E. Shonk* and George E. Boxer. Merck Institute for Therapeutic Research, Rahway, New Jersey.

Recent studies on the metabolism of triosephosphate (TP) in normal and malignant tissues stress the importance of intramitochondrial oxidation of glycerophosphate (GP) to dihydroxyacetone phosphate (DHAP). It had been assumed that the product of the mitochondrial GP-oxidase re-entered the cytoplasmic glycolytic system at the level of DHAP. It has been found that isotonically prepared mitochondria from rat liver and kidney contain TP-isomerase even after extensive washing to remove soluble, cytoplasmic enzymes. The mitochondrial TP-isomerase can be released from the mitochondria by treatment with 0.1% deoxycholate and is present in large excess over GP-oxidase. In its characteristics, K_{eq}, equilibrium, phosphate inhibition, it resembles the cytoplasmic, soluble TP-isomerase. Cellular distribution of enzyme activities involved in TP metabolism is compatible with a cyclic path for DHAP in normal, but not malignant cells, via the following reactions: 1) Cytoplasmic DHAP would be reduced to GP by DPNH formed in cytoplasm; 2) entering the mitochondria GP would be oxidized to DHAP yielding two reducing equivalents to the electron transport chain; 3) DHAP would be isomerized intramitochondrially to glyceraldehyde phosphate (GAP); 4) GAP after transfer to the cytoplasm would again yield electrons for reduction of another molecule of DHAP. (Supported by CCNSC, NCI, NIH Contract #SA-43-ph-1886.)

9 *Biochemistry*

ALLANTOIN EXCRETION IN TUMOR-BEARING RATS. Julius White, Robin H. Brooks*, Jane Nicolet Toal* and Florence K. Millar*, Nat. Cancer Inst., Nat. Insts. of Health, Bethesda, Md.

Comparative studies were made in rats bearing the Walker 256 tumor and ingesting either a 20 percent casein diet or a similar diet in which the casein was replaced by desiccated Walker 256 tumor tissue as a sole source of protein. Rats ingesting the casein diet showed a gradual increase in allantoin excretion as tumor growth progressed and reached a value of twice that observed before tumor growth was initiated. Animals ingesting the tumor tissue diet had an initial allantoin excretion of approximately twice that of animals on the casein diet. However, with increased tumor growth, there was only a slight increase in allantoin excretion (10-20 percent). Surgical removal of the tumors of animals on the casein diet, which showed elevated excretion of allantoin, resulted in an immediate reduction in allantoin excretion which fell below normal and remained subnormal for 4-8 days. Surgical removal of tumors of animals on the tumor diet also resulted in a drop in allantoin excretion but to normal values. In addition to the lowered allantoin excretion resulting from the removal of the tumor, there was an equivalent elevation in urea nitrogen.

10 *Pharmacology*

EFFECT OF CITROVORUM FACTOR ON THE INHIBITION OF FORMATE INCORPORATION IN VIVO INDUCED BY ANTIFOLIC DRUGS. Anthony W. Schrecker*, J.A.R. Mead*, Mary R. Lynch*, and Abraham Goldin, Laboratory of Chemical Pharmacology, National Cancer Institute, Bethesda, Md.

It has been shown (Goldin et al., J. Nat. Cancer Inst. 22: 811, 1959) that the therapeutic effect of daily treatment with 40 mg/kg of 3',5'-dichloroamethopterin (DCM) in mice bearing systemic leukemia L1210 was nullified by concomitant daily administration of 20 mg/kg of citrovorum factor (CF). This prompted a study of the effect of CF on the inhibition, by DCM or amethopterin, of formate-C^{14} incorporation into the acid-soluble adenine of the leukemic spleens. When CF was injected 10 minutes before, and formate 20 minutes following, the administration of DCM, the dose ratio of CF to DCM for complete reversal of inhibition was about 100. The analogous dose ratio of CF to amethopterin was about 200. Administration of CF after injection of DCM was less effective than pretreatment. Recovery from the inhibitory effect of DCM on formate incorporation was more rapid when CF was administered along with the drug. The relationship between increasing amounts of folic acid antagonists and increasing amounts of CF needed for reversal of inhibition suggests mutual inhibition of active transport across cell membranes, in addition to involvement of the dihydrofolic reductase system.

11 *Pharmacology*

DIHYDROFOLATE REDUCTASE ACTIVITIES IN TISSUES OF MICE WITH ANTIFOLATE-SENSITIVE AND ANTIFOLATE-RESISTANT LEUKEMIA. D.Misra*, S.R.Humphreys*, M.Friedkin, A.Goldin, and E.J. Crawford*. Dept. Pharmacology, Tufts Univ.Sch.of Med., Boston and Lab.Chem.Pharmacology, National Cancer Inst., Bethesda.

It is known from the works of Nichol, Welch, and Burchenal that some cells have an increased capacity to convert folic acid to citrovorum factor when resistance to folic acid antagonists develops. In the present study a single step in the overall conversion has been examined. Enzymatic analyses of extracts of acetone powders prepared from tissues of CXDBA hybrid male mice revealed the following: Spleens of normal mice had very little or no dihydrofolate reductase. Upon invasion of the spleen by antifolate-sensitive leukemic cells (L1210Z) the activity increased to approx.80 millimicromoles of dihydrofolate reduced by TPNH per mg. of protein per hr. at 28°; whereas upon invasion by amethopterin-resistant leukemic cells ($M_1$6R--a subline of the sensitive L1210) enzyme levels of approx.220 (same units as above) were found. A similar enzymatic pattern existed in the solid tumors at the site of implantation and also in the infiltrated livers. This situation can be considered as an example of drug resistance due in part to survival of cells capable of producing enough enzyme to support growth in the face of the inhibitory action of antifolate. See Fischer and Welch (Science, 126:1018, 1957).

12 *Pathology*

Proliferation of Tumor Cells in Rat Liver and its Relationship to the Transformation of Deoxycytidylic Acid. Silvio Fiala, Anna Fiala & Walter Glinsman, Dept. of Pathology, Columbia U. & Francis Delafield Hospital, New York City. Introduced by E.E.Sproul.

Massive proliferation of cells in 3'-Me,4-DAB carcinogenesis in rat liver coincides with malignant transformation. A depletion of phosphatases converting deoxynucleotides to corresponding nucleosides, due to the loss of cytoplasmic structures & reappearance of dCMP-deaminase, which disappears from tissue few days after birth, marks the onset of massive proliferation. In regard to dCMP, the carcinogenic process in rat liver is characterized by the reaction dCMP→dC in the 1st stage and by the reaction dCMP→dUMP in the 2nd. Analysis of the data shows that malignant transformation occurs with a frequency greater than is the mitotic index of the liver. The sudden and simultaneous change in many cell parameters at the critical stage indicates that every cell containing 3'-Me,4-DAB for a long enough period becomes cancerous if & when cell division takes place.

13 *Pathology*

STUDIES OF SUCCINIC DEHYDROGENASE IN NEOPLASTIC LESIONS OF THE HUMAN LARGE INTESTINE AND THE HAMSTER CHEEK POUCH. Lee W. Wattenberg, Univ. of Minn. Med. School, Minneapolis.

A morphologic histochemical study of the succinic dehydrogenase system (tetrazolium salt reduction technique) has been carried out in normal mucosa, benign adenomatous polyps, and carcinoma of the large bowel. The hamster cheek pouch at various stages of chemically-induced carcinogenesis has been investigated in a comparable manner. High succinic dehydrogenase activity has been observed in the deeper elements of atypical glands of benign polyps and also in the basal cells of some small carcinogen-induced papillomas of the hamster cheek pouch. This appears to represent a stage in the transition of these premalignant lesions to their corresponding malignant counterparts which come to have a very low succinic dehydrogenase activity. In the large bowel some elements in benign proliferative lesions also have low succinic dehydrogenase activity. The significance of the histologic relationships between these elements with low activity and the basal elements with high activity will be discussed. (Supported by Field Investigation Grant CS-9599 of the National Cancer Institute, U.S. Public Health Service)

14 *Pathology*

A CYTOCHEMICAL AND ELECTRON MICROSCOPIC STUDY OF EXPERIMENTAL SQUAMOUS CELL CARCINOMA. D. G. Scarpelli and E. von Haam, Dept. of Pathology, Ohio State Univ., Columbus, Ohio.

Squamous cell carcinoma of the uterine cervix was induced in 30 C3H female mice by the technic of Murphy (Am. J. Path. 29: 603, 1953). Both early and advanced tumors were prepared for various cytochemical studies and electron microscopy. Neoplastic cells at the apparent growing edge of tumors generally displayed large nucleoli which stained intensely for RNA and showed more cytoplasmic basophilia than did tumor cells in more quiescent areas. These cells also exhibited more intense activity of diphosphopyridine, triphosphopyridine nucleotide diaphorase and glucose 6-phosphate dehydrogenase than did cells in the more central areas of tumors. Tumor cell mitochondria showed considerable variation in size and morphology. In neoplastic cells at the growing edge of tumors mitochondria were numerous and were occasionally filamentous. In more centrally located tumor cells there were fewer mitochondria, many of which were swollen and surrounded by dense osmiophilic material. The ergastoplasm was devoid of membranes and consisted almost wholly of 30-300 Å granules. Virus-like 900 Å bodies were found occasionally in the cytoplasm of malignant cells. Increased basophilia and hypertrophic endoplasmic reticulum were present in fibroblasts adjacent to tumor cells.

249. TUMORS–Radiation Effects

1 *Biochemistry*

EFFECT OF X-IRRADIATION ON THE METABOLISM OF EHRLICH ASCITES CARCINOMA CELLS. Elmon L. Coe*, Eugene N. Garcia* and Ralph W. McKee. Dept. of Physiol. Chem., Univ. of Calif., School of Med., Los Angeles, Calif.

Tumor cells, grown 7 days in mice, were divided into two equal portions. One portion was exposed to 2000R of X-irradiation and the other was used as a control. The cells were not washed, but part of the ascites fluid was removed before irradiation and 55 mM pH 7.4 phosphate buffer was added before metabolic studies. Whole cells were incubated, 27° or 37°C for 25 min. or less, with and without glucose, and changes in various metabolites were followed. Effects of irradiation were usually evident but were often not reproducible, making generalizations difficult. Among the more consistent changes after irradiation were: (a) a lowering of endogenous lactate and triose phosphate; (b) an increase in the ratio of glycolytic rate to endogenous respiratory rate (37°); (c) a higher endogenous level of ATP and a slower decline in the ATP with decreasing oxygen concentration (27°); (d) a tendency for the ATP to decline more sharply immediately after addition of glucose. A reasonable interpretation of the data is that x-irradiation causes acceleration of glycolysis and generally increases the Michaelis constants of the endogenous ATP utilizing reactions. Indirect evidence also suggests that the phosphorylative efficiency of glycolysis is decreased.

2 *Pharmacology*

P^{32} INCORPORATION IN X-IRRADIATED Hela AND KB CELLS IN CULTURE. Kenneth W.Floyd* and R. W. Whitehead. From Depts. of Radiology & Pharmacology. Univ. of Colorado Medical Center, Denver, Colorado.

Hela and KB cells were incubated with $P^{32}O_4$ immediately after x-irradiation and the cells remaining attached to glass were harvested 24 hours later. The specific activities (cpm/10^6 cells) of the major phosphorus components were compared after fractionation by the Schmidt-Thannhauser method followed by adsorption of the organic phosphate on Norite A. Increases in the specific activities of all components were noted after x-irradiation. A comparison of the distribution of label in the fractions of the irradiated cells with those unirradiated indicates that these ratios were essentially unaltered. Comparisons of the increases in specific activities found with the maximum increase in volumes expected, suggest that radiation may be producing a stimulation of metabolism in these non-dividing cells as they become radiation giant cells. The distribution of label in radiation giants after a 24 hour incubation with $P^{32}O_4$ indicates a 3 to 4 fold increase in phospholipid metabolism. Fractionation of the cells not attached to glass reveals a population of cells with lowered metabolism. These may be responsible for the decreases in metabolism observed following x-irradiation. (Supported by grant #C-3925 of USPHS).

3 *Pathology*

EARLY MICROSCOPIC DIAGNOSIS OF LYMPHOSARCOMA IN SITU IN THYMUS OF IRRADIATED MICE. Henry S. Kaplan, (Dept. of Radiology, Stanford University School of Medicine, Stanford, Calif.)

A "nascent" stage of thymic lymphosarcoma in situ is often recognizable at about 100 days after irradiation of C_{57}BL mice (W.H. Carnes and H.S. Kaplan, Proc. Am. Ass'n Cancer Res. 2:99, 1956). In groups of C_{57}BL mice sacrificed at 50 and 100 days after 4 doses of 200r at 7 day intervals, a positive microscopic diagnosis was made in 36 of 43 (84%) and 35 of 36 (97%), respectively. Final incidence at 250-300 days in identically irradiated simultaneous controls was 53 of 57 (93%) and 35 of 37 (95%), respectively. In another experiment, transthoracic thymic biopsies have been taken at 25, 50 or 75 days after either whole-body or thigh-shielded irradiation. The biopsied animals were then maintained without further treatment. The biopsies were read "blind" under code numbers, but were grouped with respect to time of biopsy. No diagnosis was possible at day 25 (4 days after last irradiation) but overall diagnostic accuracy at days 50 and 75 was 81% and 79%, respectively. Biopsy at 25 days (but not at 50 or 75 days) significantly interfered with lymphoma development, as compared with final incidence in unbiopsied irradiated controls. Microscopic appearance of the several stages will be illustrated and diagnostic criteria discussed.

4 *Pathology*

METASTATIC SPREAD OF LYMPHOSARCOMA AS INFLUENCED BY RADIATION. M.R. Sikov, B.B. Cook* and J.E. Lofstrom*. Dept. of Radiol, Wayne State Univ. Coll.of Med. and Rec. Hosp., Detroit, Mich.

The use of a component of systemic radiation in conjunction with local tumor radiotherapy has been shown to inhibit tumor growth more than direct exposure of the tumor. This was reinvestigated in terms of the incidence of metastases, complete regression, and regrowth of tumor in the L-1 Lymphosarcoma. A tumor dose of 800 r increased cure rate as well as the frequency of metastases; whole body radiation decreased metastases but did not alter cure rate. Approximately 70% of the tumors treated with 1400 or 2000 r metastasized, with or without systemic exposure. Higher doses of total body radiation increased the fraction of animals completely cured. An attempt was made to simulate the oncolytic effects of whole body radiation by injecting plasma from previously irradiated rats. Plasma obtained at times ranging from 10 minutes to 5 days after 1000 r whole body was found to be without effect. It was concluded that irradiation of this tumor increases the frequency of metastases. At lower tumor doses, whole body radiation tends to prevent the development of such secondary lesions; this was not true at higher doses. Higher doses of systemic exposure also increased the fraction of animals in which complete cure was obtained. (Supported by Grant from American Cancer Society.)

250. VIRUSES

1 *Biochemistry*

UTILIZATION OF HeLa CELL COMPONENTS AS PRECURSORS FOR THE SYNTHESIS OF ANIMAL VIRUS NUCLEIC ACID. N. P. Salzman and E. D. Sebring (intr. by H. D. Baernstein.) Natl. Inst. of Health, Bethesda, Md.

Short-term exposure of HeLa cells to radioactive nucleic acid precursors (adenine-8-C^{14} and uridine-2-C^{14}) results in a high degree of labelling of the free cellular nucleotides and a much lower labelling of the RNA nucleotides. Cells labelled in such a manner were infected with poliovirus and the specific activities of the nucleic acid bases in RNA, in the acid soluble fraction of the cell and in the purified poliovirus (Levintow and Darnell, Fed. Proc., 18, 273 (1959)) were determined. The data establish that the acid soluble fraction of the cell is the sole source of viral RNA, and that there is no significant direct utilization of the cellular RNA for virus synthesis. These data and earlier studies by Eagle and Habel (J.Exptl.Med., 104, 271 (1956)) suggest that cells normally contain a full complement of the small molecular weight components required for poliovirus synthesis.

In contrast, the synthesis of vaccinia virus requires the continuing synthesis by the cell of a viral component, thymidylic acid. The addition of the inhibitor 5-fluoro-deoxyuridine, which blocks thymidylic acid synthesis, prevents vaccinia virus synthesis.

2 *Biochemistry*

RELATIONSHIP BETWEEN THE TIME OF SYNTHESIS OF VIRUS PROTEIN AND VIRUS NUCLEIC ACID IN POLIOVIRUS INFECTED HeLa CELLS. L. Levintow and J. E. Darnell, Jr.* Natl. Insts. of Health, Bethesda, Md.

The development of a convenient method for purifying poliovirus and the fact that the soluble pool of the cells contributes the amino acids and nucleotides for the synthesis of virus protein and RNA, permit determination of the time of synthesis of the latter two constituents in relation to the appearance of mature virus. A radioactive precursor was added at intervals to aliquots of an infected culture, purified virus was isolated from each of the aliquots, and a specific viral constituent isolated and assayed for radioactivity. Those viral macromolecules already formed when the label was added would not be radioactive. With valine as a precursor, it has been established in this manner that virus protein synthesis begins about the same time as does maturation, but is completed 1-1.5 hours before maturation. The best precursor for the study of virus RNA synthesis was adenosine-8-C^{14}, which equally labels AMP, ADP and ATP of the cell pool within 15 minutes, although maximal labeling requires a somewhat longer time. Experiments with this precursor indicate that the bulk of the virus RNA is formed no earlier than the protein. The results are consistent with the view that immediately after its formation, the RNA is incorporated into mature virus.

3 *Pathology*

A FACTOR PRESENT IN ADENOVIRUS SUSPENSIONS CAPABLE OF MODIFYING ERYTHROCYTE RECEPTORS. Julius A. Kasel, Wallace P. Rowe, and John L. Nemes (intr. by R. E. Ritts, Jr.) Natl. Insts. of Health, Bethesda, Md. and Georgetown Univ., Washington, D. C.

Five adenovirus types (8, 9, 10, 13 and BAR-2) agglutinate human group O erythrocytes. Treatment of O cells with *V. cholerae* receptor destroying enzyme abolishes their agglutinability by these five adenoviruses. Treatment of O cells with adenovirus types 1, 4, and 15, which do not agglutinate O cells, markedly reduced the subsequent agglutinability of these cells for three of the five adenovirus types (10, 13, and BAR-2), but not for three myxoviruses or polyoma virus. Adenovirus types 5, 7, or 17 did not alter the receptor sites for any of the five adenoviruses. Most of the infectious virus of a type 15 suspension could be removed by adsorption onto rat erythrocytes without reduction in titer of the erythrocyte receptor modifying (ERM) factor in the remaining suspension. ERM activity was inhibited by homologous rabbit antiserum but not by control rabbit antiserum. Six exposures of type 15 suspension to fresh O cells did not affect ERM titer. The results indicate that a factor in tissue culture preparations of certain adenovirus types, separable from the infectious particle but containing specific antigens, is capable of modifying erythrocyte receptors, possibly by enzymatic action.

4 *Pathology*

IMMUNOLOGICAL RELATIONSHIPS OF MEASLES, DISTEMPER AND RINDERPEST. David T. Imagawa and John M. Adams. Depts. of Pediatrics and Infectious Diseases, School of Medicine, Univ. of Calif., Los Angeles, and Vet.Admin.Hosp., Long Beach.

Serum samples from measles, distemper and rinderpest were analyzed for measles antibody by neutralization tests in tissue culture and for distemper antibody by neutralization tests in suckling mice. The results present further evidence of an antigenic relationship among these 3 viruses. Studies with 10 pairs of acute and convalescent sera from patients with measles showed significant increases to distemper and measles antibodies. The response to the two antibodies showed a close parallelism. Sera from guinea pigs, rabbits, monkeys and ferrets immunized with measles virus contained distemper antibody in low dilutions. Ferrets immunized with avian or mouse-adapted strains of distemper virus and subsequently challenged with virulent distemper virus demonstrated neutralization of measles virus in low dilution when the response to distemper antibody was good. Measles antibody response was absent or poor in direct relationship to distemper antibody level. Sera from guinea pigs following inoculation with distemper virus showed a very poor response to distemper and none to measles. Rinderpest antisera (kindly furnished by Dr.P.Goret of France) contained measles and distemper antibodies, whereas, normal beef sera failed to neutralize either virus.

5 *Pathology*

ELECTRONMICROSCOPIC STUDY OF CANINE DISTEMPER INCLUSIONS. Jutaro T. Tawara*, Joseph R. Goodman*, David T. Imagawa and John M. Adams. Depts. of Pediatrics and Infectious Diseases, School of Medicine, Univ. of Calif., Los Angeles, Vet. Admin. Hosp., Long Beach, and Okayama Univ., Japan.

Two types of inclusion-like bodies are recognized. The first type is the classical eosinophilic body which under electronmicroscopy is very osmophilic and dense, and thus reveals no fine structure. The second type of inclusion appears to be composed of matrix in the early stages of its development, and particles of approximately 120 millimicron in the latter stages. Ferrets inoculated with virulent distemper virus are sacrificed at various intervals in order to obtain infected bladder epithelium. The identity of the dense inclusion bodies on the electronmicrographs is established by staining corresponding sections with hematoxylin and eosin. Adjacent thick and thin sections are mounted for light and electron microscopic study. The thick section is treated with acetone and xylol to remove methacrylate and then is stained with hematoxylin and eosin. The thin sections are processed for the electron microscope.

6 *Pathology*

RESPONSE OF CILIATED AND MUCOUS EPITHELIUM TO NEWCASTLE DISEASE VIRUS. F. B. Bang and M. Foard*. Johns Hopkins University School of Hygiene and Public Health, Baltimore, Md.

The initial phases of infection of cells in intact chick trachea, organ culture of trachea, and of trypsinized chick embryonic cells have been compared by plaque counting techniques. Infection of roller tube cultures of trypsinized cells with Cg strain showed increasing numbers of infected cells with time when virus was left in contact. In contrast, the differentiated epithelium both in organ culture and in trachea obtained directly from the chick, showed a marked drop in the number of infected cells and free virus 4 to 7 hours after infection. This occurred despite the continued presence of virus and is attributed to a sloughing out of differentiated cells when they are infected, and to a continued protection by mucous. Cells from all three situations liberated infectious virus when treated with trypsin after previous treatment with antibody and thorough washing. Suspension of the cells in water was used to kill cells and thus test for virus alone.

7 *Pathology*

CORRELATED MICROSCOPIC AND MICROSPECTROPHOTOMETRIC STUDIES OF DNA OF TISSUES FROM MICE AND HAMSTERS INFECTED WITH POLYOMA VIRUS. Cecilie Leuchtenberger, Sarah Stewart*, Rudolf Leuchtenberger* and Bernice Eddy*. Children's Cancer Research Foundation, Boston, Mass., Western Reserve University, Cleveland, Ohio, and Nat. Inst. of Health, Bethesda, Md.

This study is concerned with the sequence of events occurring in cells after infection of newborn mice and hamsters with polyoma virus. Particular attention was focused on the cytopathotoxic and proliferative effects of polyoma virus in relation to DNA content of cells. Cytopathotoxic changes preceded cell proliferation and tumor formation, whereby the pathotoxic changes were observed in cells which did not seem to participate in the proliferative processes including tumor formation. For example, in the kidneys of mice, ballooning of cells and "inclusion bodies" were found only in the tubular epithelial cells while proliferation leading gradually to sarcoma formation arose in stroma cells or in lining epithelia of renal convoluted tubules continuing only to adenomas, never to carcinomas.

The DNA content of the ballooned cells was similar to that of homologous cells from non-infected controls while the DNA content of the proliferating and tumor cells was increased in accordance with the DNA synthesis occurring during mitosis. (Supported by NIH Grant No. C-4472 Path).

8 *Pathology*

CONCERNING THE NATURE OF THE INDUCTION OF ARGINASE BY THE SHOPE PAPILLOMA VIRUS. Stanfield Rogers. The Univ. of Tenn. Mem. Res. Center, Knoxville, Tenn.

Experiments indicating that the Shope papilloma virus induces an enzyme new to rabbit epithelium were recently reported (Nature 183, 1815-1816, 1959). This raised the question of whether the information for synthesis of the enzyme was derived from virus DNA or whether from rabbit information. Should the arginase be derived from virus information, one might expect that it would be antigenic. A factor has been demonstrated in the serum of animals carrying the papilloma which yields a precipitin reaction with extracted arginase even from the individual rabbits own tumors. The occurrence of this precipitin is associated with a relative loss of arginase activity in the supernate following centrifugation. Both the Vx-7 and Vx-2 carcinomas derived originally from virus induced papillomas contain this arginase. (Supported by a grant from the Jane Coffin Childs Memorial Fund.)

9 *Pathology*

RESPONSES OF TWO HUMAN STRAINS OF EPIDERMOID CARCINOMA(CERVIX) GROWN ON COLLAGEN TO E.E.E. VIRUS. G. O. Gey*, F. B. Bang, M. Svotelis*, and M. Foard*, Johns Hopkins Medical Institutions Baltimore, Maryland.

We have followed the growth and destruction by Eastern Equine Encephalomyelitis Virus resulting in chronic infections up to 3 years but differing in duration and destruction in the two cervical carcinoma strains. HeLa cultures grown on reconstituted rat-tail collagen show a greater accumulation of damaged and destroyed cells following virus growth than those grown on glass. In contrast the ElCu strain shows little accumulation and clears more rapidly. Previous treatment of cells with trypsin allowed a more destructive response in the HeLa cultures. This may be explained by release of cell cohesions permitting the rounded naked cells still attached to collagen to be more effectively exposed. Repeated challenges of chronically infected cultures with 10^8 and 10^5 L.D. 50's demonstrated marked resistance. Cells grown in media supplemented with N.C.T.C. 109 gave more rapid growth than in the 50% cord serum but did not alter the chronicity of the infection.

(Supported by grants from the U.S.P.H.S. and The American Cancer Society.)

10 *Immunology*

EFFECT OF OXIDATIVE CONTROLLED POTENTIAL ELECTROLYSIS ON VIRUSES. J. V. Hallum* and J. S. Youngner, Univ. of Pittsburgh, School of Medicine.

When virus suspensions are subjected to oxidative electrolysis at a fixed potential, a loss of infectivity is observed. This loss of infectivity is not accompanied by a loss of hemagglutinin (HA) or antigenic properties if the applied potential is controlled. For example, treatment of allantoic fluid infected with influenza virus (Lee) at 1.35-1.45 volts resulted in complete loss of infectivity within 4 hours without any loss of HA, while treatment at 1.45-1.55 volts caused a complete loss of HA. Rate of loss of infectivity is dependent on the non-viral materials present, e.g., a poliovirus sample which required 43 hours at 1.4 volts for inactivation, required only 6½ hours after purification by one cycle of ultracentrifugation. The rate of reaction is shown to be dependent on the applied potential and on contact of the virus with the working platinum electrode. No substances are produced during electrolysis which affect the infectivity of the virus. Influenza viruses (A,A',B), NDV, poliovirus, adenovirus, vaccinia and polyoma viruses have been studied. The rates of inactivation by oxidative electrolysis obtained with the different viruses show that inactivation of each virus has a characteristic potential dependence.

11 *Immunology*

INTRAUTERINE INFECTION OF POLYOMA VIRUS C. P. Li, and W. G. Jahnes Div. of Biologics Standards, Natl. Insts. of Health, Bethesda, Md.

Experiments were conducted to see whether mice can be infected with polyoma virus in utero. Adult female mice were in-fected either before or after mating or during early pregnancy with approximately $10^{4.0}$ $TCID_{50}$ of polyoma virus in tissue culture (TC) fluid by intracerebral, intramuscular or intraperitoneal injection. From 5 to 29 days after inoculation some pregnant mice were sacrificed and their embryos trypsinized for preparation of monolayer TC in roller tubes, using Eagle's medium with 10% calf serum for growth and the same medium with 2% calf serum or horse serum for maintenance. Cultures were observed for about 2 months and TC fluid was harvested from time to time to test for polyoma virus by hemagglutination reaction. If positive, virus was identified by hemagglutination inhibition (HI) test with anti-polyoma virus serum and by tumor production in 1-day-old hamsters. Out of 10 such experiments, polyoma virus was isolated 7 times. Contamination from mother's blood was unlikely because it often showed a high antibody titer as revealed by HI test. Pregnant mice not sacrificed were allowed to deliver their litters and young mice were bled for HI test at the age of 4 weeks. More than 50 young mice were tested, but none showed antibodies.

12 *Immunology*

EFFECT OF VACCINIA INFECTION ON CERTAIN CARBOHYDRATE METABOLIZING ENZYMES IN "L" CELLS. Victor B. Slotnick, Dept of Microbiology, Hahnemann Medical College, Philadelphia, Pa. (intr. by B.A. Briody)

Enzyme assays were performed on normal and vaccinia-infected "L" cells grown in monolayer or fluid suspension. The activity of glucose-6-phosphate, 6-phospho-gluconic acid, lactic acid, isocitric acid and malic acid dehydrogenases were measured using intact, freshly harvested "L" cells. By suspending "L" cells in 70% glycerol, light scattering was reduced to a minimum allowing direct spectrophotometric measurement of triphosphopyridine nucleotide reduction or reduced diphosphopyridine nucleotide oxidation at 340 mu. Nitrogen analysis and virus titrations were also carried out on aliquots of normal and vaccinia-infected cultures. When a virus: cell ratio of 5:1 was used, the activity of glucose-6-phosphate and 6-phospho-gluconic acid dehydrogenases was decreased and protein synthesis and virus multiplication were not observed. Using a virus:cell ratio of 1:1 the activity of all the enzymes investigated was increased within 12-24 hours post infection. At 36 hours, the infected cultures showed decreased activity of all the enzymes as compared to uninfected cell cultures. In addition, increased nitrogen content and virus titers were observed within infected cultures, suggesting that protein synthesis and viral multiplication had occurred.

13 *Immunology*

INTERFERING EFFECT OF SODIUM DESOXYCHOLATE-TREATED MAYARO VIRUS. J.R. Henderson* and R.M. Taylor. Yale University School of Medicine, New Haven, Conn.

Experiments were designed to determine the course of Sindbis virus multiplication in cell cultures previously inoculated with sodium desoxycholate (SDC)-treated and untreated Mayaro virus. Differences in time of plaque development in chick embryo monolayer cultures were used to distinguish between these viruses in the same culture. The data indicate that Sindbis (challenge virus) multiplication decreased in an approximately exponential manner as Mayaro (interfering virus) adsorption time and infection multiplicity increased. When SDC-treated Mayaro virus was used as the interfering agent, Sindbis multiplication was restricted by much the same rate as when untreated Mayaro was used as the interfering agent. This interfering effect of SDC-treated Mayaro could not be demonstrated in the presence of RNAase. It is suggested that interference in this virus system may be directed by certain ribonucleic acid-like components which become exposed after SDC treatment. (Supported in part by a grant from the Army.)

14 *Immunology*

CHARACTERISTICS OF A PERSISTING FILTRABLE AGENT IN A CONTINUOUS CULTURE OF MOUSE CELLS.** H. F. Maassab, Dept. of Epidemiology, School of Public Health, Ann Arbor, Michigan.

A continuous culture of embryonic mouse cells was established in this laboratory and has been subcultured over 100 times. Definite morphological changes were observed under certain nutritional influences and with prolonged cultivation. The altered morphology persisted upon serial passage and was shown to be due to a virus. Viral activity could be demonstrated when either the altered cells or the media is used to infect adult mice, primary chick kidney or human amnion cell cultures. The changes induced by the virus in these hosts differed markedly. An additional finding was that the altered cells could be induced to degenerate when subjected to various stresses, such as light and frequent changing of the medium. Data will be presented on the relationship of the virus to other viruses and especially to those of mice.

**This investigation was conducted under the auspices of the Commission on Influenza, AFEB, and was supported by the Office of the Surgeon General, U.S. Army, Washington, D.C.

15 *Immunology*

DEMONSTRATION OF VIRUS ANTIGEN IN HUMAN POST MORTEM TISSUE BY FLUORESCENT ANTIBODY TECHNIQUE. John L. Riggs* and Gordon C. Brown, Dept. of Epidemiology and Virus Laboratory, School of Public Health, University of Michigan, Ann Arbor, Michigan.

Although some bacterial antigens have been demonstrated in paraffin sections by the fluorescent antibody technique, this procedure has not been generally applied to the identification of viral infections. The successful application of this technique is illustrated by the results herein described. Two neo-natal deaths were proved to be caused by Coxsackie B-2 and B-3 respectively by identification in tissue cultures of virus recovered from fresh heart, liver, lung, pericardial fluid and intestinal contents of one infant and the heart of another. Tissues had also been fixed in formalin at autopsy. The blocks were washed in water to remove HCHO, dehydrated, and impregnated with low melting point paraffin. Sections were then cut and mounted, cleared of paraffin, hydrated, then stained with homologous and heterologous anti-Coxsackie B monkey sera labeled with fluorescein isothiocyanate and absorbed with monkey liver powder to reduce non-specific staining. Sections were examined under UV light and type specific fluorescence was observed. Extension of this diagnostic technique to other post mortem, stool, and tissue culture specimens is being investigated.

16 *Immunology*

ALTERATION IN COMPOSITION OF NUCLEAR RNA FROM HAMSTERS WITH EQUINE ABORTION VIRUS (EAV) HEPATITIS. Glenn A. Gentry* and Charles C. Randall. U. of Miss. School of Med., Jackson.

Previous studies have suggested that infection of hamsters with EAV produces alterations in the nucleic acid metabolism of parenchymal liver cell nuclei. In the present study the base composition of DNA and RNA from citric acid-isolated nuclei was determined. No changes were found in the base composition of DNA, but with RNA the proportion of guanylic acid increased, with a decrease in adenylic acid, at 12 hours after inoculation. No changes were noted in net nuclear content of RNA or DNA. Stained smears of the isolated nuclei indicated that cytoplasmic contamination was well under 1% in both normal and infected preparations, and that the heterogeneity of the liver cell population probably was not an important factor. Since it is known that dilute citric acid will extract certain fractions of RNA from nuclei of other animals, the present data probably are representative of a fraction of the nuclear RNA rather than the total. Although a complete interpretation must await further studies, it is suggested that the findings are the reflection of a shift in heterogeneous fractions or in composition of host RNA, or the presence of a new abnormal RNA, rather than an accumulation of virus particles. (Supported by a grant from the N.I.H.)

17 *Immunology*

THE ROLE OF ANTIBODY IN THE PERSISTENCE OF HELA CELL CULTURES INFECTED WITH ADENOVIRUS TYPE 2. Ann Schluederberg (Introduced by Bernard Roizman) Department of Microbiology, The Johns Hopkins University, Baltimore, Md.

Human immune γ globulin added to culture fluid 4 hours after exposure of HeLa cells to concentrated Adenovirus type 2 had no effect on the rate of intracellular accumulation of virus or on the time of degeneration of the cells. From 300 to 500 ID_{50} of intracellular virus per cell were recovered. The virus used in these experiments was separated from a cytotoxic factor by ultracentrifugation in a density gradient. Human or guinea pig immune sera or human immune γ globulin added to cultures together with known numbers of infected cells were able to delay but not prevent the ultimate destruction of the cell population. Gamma globulin absorbed with inactivated virus free of cytotoxic activity was less effective. There was a reduction in the neutralizing but not the anti-cytoxic activity of the γ globulin following absorption. A relationship was demonstrated between the number of cells initially infected, neutralizing antibody levels and the time at which the cells degenerated. It is concluded that antibody enables infected HeLa cultures to persist longer by suppressing the spread of virus from cell to cell. (Supported by a grant from USPHS # E 1739.)

18 *Immunology*

RELATIONSHIP BETWEEN ANTIBODIES AND IMMUNITY IN MEASLES. Francis L. Black, and S.R. Sheridan.* Yale University School of Medicine, New Haven, Conn.

It is generally agreed that humoral antibodies form a major form of defense against systemic viral infection. However, it has rarely been possible to determine the extent to which the presence of antibodies actually correlated with individual immunity or to estimate the minimal titer necessary for protection. Measles is a singular homogeneous disease in terms of the original susceptibility of the host, freedom from re-infection and absence of effective cross protection from antibodies to other agents. Outbreaks of measles in an institution where serum specimens had been collected previously furnished an opportunity to correlate the pre-epidemic antibody titers with the effects of exposure to the virus. Neutralizing antibody titers as low as 4 were correlated with immunity. Although a few children with titers below this level were also immune most persons with lower titers proved susceptible to infection. Some of the children with complement fixing antibody titers of 4 or 8 were susceptible but no one with a higher CF titer became infected. Very few individuals with prior neutralizing antibodies showed a boost in titer during the epidemics.

19 *Immunology*

LATENT POLYOMA VIRUS INFECTION OF MOUSE FIBROBLASTS IN TISSUE CULTURE. Harold E. Pearson. Dep't Public Health, Univ. South. Calif. School of Med. and Lab. Divis., Los Angeles Co. Hosp., Los Angeles, Calif.

Polyoma virus of mice is readily propagated in tissue cultures of mouse embryo fibroblasts. According to previous reports (Proc.Soc.Exper.Biol.and Med. 1958, 98, 848; J.Exper.Med. 1959,109,379) cytolysis occurs after 4-32 days of incubation. We have maintained continuously in tissue culture in Eagle's solution a strain of mouse fibroblasts originally obtained from a peritoneal exudate in an albino (Swiss) mouse. Infection of these uncloned cells with a polyoma virus clone is followed in 12-14 days by cytolysis of most cells. The hemagglutination titer of supernatant fluid at this time for guinea pig cells is 512 and the tissue culture titer is 10^{-5} - 10^{-6}. The few scattered tissue culture cells which survive despite viral activity continue to grow and soon have a comparable growth rate and the same general appearance in fresh and stained preparations as noninfected cells. However, virus is readily obtained from the supernatant fluid of such cultures for at least several months. (Aided by a grant from the Nat. Insts. of Health)

20 *Immunology*

STUDIES ON ISOLATED VIRAL INCLUSIONS. Charles C. Randall and L. G. Gafford*. Univ. of Miss. School of Medicine, Jackson.

Thirty years ago Goodpasture indicated that Fowlpox inclusions could be liberated by trypsin. In the present study the feasibility of obtaining quantities of inclusions for analysis was explored. Trypsin digests of chick skin were more satisfactory than chorioallantois. Quantities of relatively pure inclusions were prepared by centrifugation. Smears were examined by special stains. The inclusions contained neutral fat and protein and were negative for carbohydrate. Following DNA ase the inclusions were Feulgen negative whereas hydrolyzed controls were positive. Squashed inclusions were packed with elementary bodies. Other samples were enumerated and lyophilized for analysis. The dry weight varied from 4.50 - 5.70 mg x 10^{-7} per inclusion. The dry residue following lipid extraction contained 23% protein. Partition of the dissolved lipid was determined as total lipid, free fatty acid, glycerol, phospholipid, total and esterified cholesterol. Total lipid averaged 50% of the dried weight of inclusion material. Significant comparison with infected epithelium reveals that inclusions contain a much greater concentration of total and esterified cholesterol and 3/4 less phospholipid. These studies indicate that inclusions offer a unique source of material for the study of the inclusion matrix and virus. (Supported by a grant from NIH.)

21 *Immunology*

MORPHOLOGY OF THE VACCINIAL INFECTION IN TISSUE CULTURE. M. Alousi*, H. Kurtz* and W. W. Ackermann, Dept. of Epidemiology, Univ. of Michigan, Ann Arbor.

A sequence of chemico-morphologic alterations induced by vaccinia virus in cultures of HeLa cells has been observed. The cultural responses concern the organization of cells relative to one another, as well as the modification of subcellular elements. The intensity of response, prominent in both nucleus and cytoplasm, is partially determined by the concentration of inoculum and temperature. Alterations are evident within one hour, yet much cell structure and organization remain by 24 hours. Certain cellular changes are autoreversing during the early part of the infectious sequence, e.g. nuclear and cytoplasmic contraction. Nearly all cells develop multiple ovoid, acidophilic, cytoplasmic inclusions the size of nucleoli. These are Feulgen positive. Their evolution and significance will be discussed. (Supported by a grant from The National Foundation)

22 *Immunology*

FLUORESCENT ANTIBODY STUDIES ON THE PATHOGENESIS OF WEST NILE VIRUS. W. D. Kundin*, Chien Liu, & Te Yong Lou* Section of Virus Res., Univ. of Kansas School of Med., Kansas City, Kans.

The pathogenesis of West Nile virus was studied by fluorescence microscopy. Young chicks were infected subcutaneously and suckling mice were infected intracerebrally and intraperitoneally. Frozen sections were cut from various tissues and stained with fluorescent antibodies. Infected chicks developed an inapparent infection, while suckling mice invariably died of a fulminating infection. In chicks, viral antigen was seen in the smooth muscles of the gizzard and the heart from the second day through the seventh day of infection. Virus was found less consistently in the smooth muscles of the proventriculus, esophagus, crop and pancreas. No viral antigen was observed in other tissues such as the brain, spinal cord, liver, spleen and kidney. There appeared to be a direct correlation between the presence of viral antigen and the infectious virus in tissues. In infected suckling mice, preliminary studies revealed extensive involvement of various organs as a result of virus infection in contrast to the limited infection in chicks. There was massive involvement of the central nervous system in such mice regardless of the route of inoculation. (Supported by USPHS Grant E2514).

23 *Immunology*

"TRACHOMA VIRUSES" ISOLATED IN CALIFORNIA. E. Jawetz, L. Hanna*, P. Thygeson* and C. Dawson*. Univ. California Med. Center, San Francisco, Calif.

"Trachoma viruses" have been isolated recently in Africa and Asia. We succeeded in growing similar viruses from active trachoma in California. Conjunctival scrapings collected in broth-saline with 4mg/ml streptomycin were injected into yolk sacs of 7-day embryonated eggs. After 1 to 3 blind passages embryos died on 6th to 9th days of incubation and elementary bodies abounded in yolk sac smears. Egg LD50 reached 10^{-6} in 6 more passes. The agents fixed complement (C-F) with antisera to psittacosis virus, had toxic effects, and maintained infectivity when lyophilized or stored at -40C. One strain (Bour) was isolated from acute trachoma in a California resident who had a C-F titer of 1:64 to psittacosis-LGV group antigen. This virus diluted 10^{-3} produced intense follicular conjunctivitis with abundant inclusions in M. cynomolgus. The second strain (Asgh) was isolated from the relapse of a man who acquired trachoma many years ago in Pakistan, was free from symptoms for the last 5 years, and now has a C-F titer of 1:4 to the group antigen. That virus diluted 10^{-1} produces only mild follicular conjunctivitis with rare inclusions in monkeys. Thus "trachoma viruses" may exhibit different biological characteristics, perhaps determined by source of infection or host reaction.

24 *Immunology*

THE FATE OF POLIOVIRUS ADSORBED TO HeLa CELLS. W. K. Joklik and J. E. Darnell, Jr. (intr. by Harry Eagle.) Natl. Insts. of Health, Bethesda, Md.

When P^{32}-labelled poliovirus (purified by passage through an ECTEOLA-column and CsCl density gradient centrifugation (Levintow and Darnell, J. Biol. Chem., in press) is added to S3 HeLa cells grown in suspension culture, over 95% of infectious virus is adsorbed according to first order kinetics, whereas less than one-third of the label disappears from the medium. This phenomenon was further studied by determining the fate of virus adsorbed for 5 minutes only. At both high and low multiplicities (1000-2000 and 1-2 particles per cell) 50-60% of the label spontaneously elutes at 37°; at 0° there is no elution. Eluted material has the following properties compared with the starting virus: its specific infectivity is very much lower, its density as determined in a CsCl density gradient is the same, and it adsorbs and elutes from cells with the same kinetics. Virus material which does not elute from the cells is largely degraded. After 2 hours about two-thirds of it have been converted to acid-soluble material, while the rest is made up of roughly equal amounts of RNase-resistant and RNase-hydrolyzable material which is deposited by 2 hours centrifugation at 40,000 rpm. A small amount of RNase-hydrolyzable material appears in the medium.

XUM

25 *Immunology*

STUDIES ON CANINE DISTEMPER VIRUS IN TISSUE CULTURE. Robert H. Bussell (intr. by David T. Karzon.) Dept. of Pediatrics and Dept. of Bact. & Immunol., Univ. of Buffalo School of Med.

Canine distemper virus (CDV) was shown to produce cytopathic changes in chick embryo tissue culture (TC) (Karzon and Bussell, Science 130:1708, 1959). Study of viral growth in chick fibroblast monolayers indicated that most of the viable virus was cell associated, with smaller amounts present in the supernatant fluid. Inoculation of limiting dilutions of virus into TC maintained under fluid medium resulted in formation of microplaques. This was followed by the appearance of secondary plaques with slow progression to generalized involvement. During early TC passages, the TC titer was in the same range as that obtained on the chick embryo chorioallantoic membrane (CAM). After the 30th TC passage the ability of the virus to produce lesions on the CAM was markedly reduced. Under agar overlay, small plaques were formed. After 5-6 days, the plaques were approximately 0.2 mm. in diameter, attaining a size of 2-3 mm. by the 12th day. The composition of the overlay medium influenced plaque size and appearance. A linear relationship was demonstrated between virus dilution and the number of plaques formed. The cytopathic effect and plaque formation also provided the basis for quantitative neutralization procedures.

26 *Immunology*

PURIFICATION AND IMMUNOCHEMICAL CHARACTERIZATION OF ADENOVIRUS ANTIGENS. W. Wilcox* and H. S. Ginsberg. Western Reserve Univ., Cleveland, Ohio.

Disruption of adenovirus-infected HeLa cells yields, in addition to newly synthesized infectious virus, a common complement-fixing antigen and a toxin-like material. Physicochemical, chemical and immunological studies were undertaken to study the relationship of these soluble products of infection to viral synthesis and mechanisms of cell injury. Fractionation of crude infected cell extracts was accomplished by means of adsorption to cellulose-DEAE, followed by step-wise elution from this anion exchange resin. By means of this technique each of the major products of infection, i.e., common complement-fixing antigen, soluble toxin and infectious virus, was obtained in distinct and widely separated fractions. This procedure served to isolate these products in antigenically homogeneous form, to reveal physicochemical differences among products of infection by a single virus, to indicate that antigens of type 5 adenovirus have physicochemical characteristics different than similar antigens of type 4 virus, and to distinguish these antigens from normal cell components. The implications of these findings will be discussed.

27 *Immunology*

CHARACTERISTICS OF TYPE 8 ADENOVIRUS. W. A. Clyde, Jr.* and F. W. Denny, Jr. Western Reserve U., Cleveland, O.

It has been suggested that certain properties of type 8 adenovirus are unique compared with previously studied types (Jawetz et al., Am. J. Hyg. 67, 276, 1958), but low infectivity titers produced in most tissue lines make data difficult to interpret. By use of primary human amnion cell cultures some of these problems have been overcome. Infectivity titers approximating those of other adenovirus types (10^{-3} to 10^{-5} TCD_{50}/ml) could be produced by prolonged incubation or by inoculating cultures which had first been aged. Quantitative neutralization data showed that the slope of the neutralization line approached 1 (0.86). Sequential cytopathologic studies with light, phase-contrast and fluorescence microscopy revealed that the primarily intranuclear changes begin with ringed inclusions, progress through central networks of small eosinophilic crystals, and conclude with large peripheral hexagonal basophilic crystals. By virtue of neutralization and cytopathological features, type 8 adenovirus is thus related to types 3, 4, 7 and 14, and distinguished from the subgroup composed of types 1, 2, 5 and 6.

28 *Immunology*

CHROMATOGRAPHIC FRACTIONATION OF RNA FROM MICROSOMES AND pH 5 ENZYME OF LEUKEMIA CELLS. Hilton B. Levy and Richard Lynt, Jr. (intr. by Karl Habel.) National Institutes of Health, Bethesda, Md.

Previous work from this laboratory has shown that the spleens of mice infected with Friend's murine leukemia virus incorporate administered P^{32} more rapidly than do spleens from uninfected mice. This effect can be observed early in the infection, even before the spleens begin to enlarge and when little or no pathological alteration can be seen. It also has been shown that at this early stage of the disease the increased P^{32} uptake is restricted mostly to the lipid and RNA of the microsomal and subparticulate elements. The present report deals with fractionation on ECTEOLA of RNA's from microsomes and pH 5 enzyme from infected and control spleens. The chromatographic profile of infected and control RNA's are essentially identical with respect to the amount of RNA in each fraction. The specific radioactivity of the high molecular weight RNA's from the infected tissue is higher than those from controls, while that of the lower molecular weights is equal to or lower than that of the controls. All the RNA's are heterogeneous, as shown by the fact that the chromatographically isolated subfractions have differing specific radioactivities.

29 *Immunology*

SEROLOGICAL RESPONSES IN BANGKOK HEMORRHAGIC FEVER. W. McD. Hammon, Gladys Sather* and Ambhan Dasaneyavaja.* Grad. School of Public Health, Univ. of Pittsburgh, Pittsburgh, Pa.

A combination of CF and NA tests were made on paired sera from children with a clinical syndrome diagnosed as hemorrhagic fever occurring in Thailand in 1958. This was an epidemic with 2,500 cases and 250 deaths. Acute phase sera yielded several strains each of dengue type 2 and chikungunya viruses, the former also from Aedes aegypti. Antibody rises occurred regularly to the virus type isolated and frequently also to the second though immunologically unrelated virus. Many without virus isolation developed titer rises to dengue or chikungunya or both. Frequently CF antibodies were present or developed simultaneously to several group B viruses and in such the dengue rise was very rapid and high. Chikungunya CF rises were relatively slow and NA rises rapid. Rises to both viruses might well have resulted from simultaneous or almost simultaneous infections. Since only one clinical syndrome was observed, interpretation of etiology is difficult, particularly since neither virus is normally associated with illness other than dengue or one that is dengue-like. (Conducted under sponsorship of Commission on Viral Infections, Armed Forces Epidemiological Board and supported by the Office of The Surgeon General, Department of the Army.)

30 *Immunology*

FORMATION OF POLIO AND COXSACKIE VIRUS IN FL CELLS. Jørgen Fogh and Donald C. Stuart, Jr. (intr. by Rachel Brown.) Div. Laboratories and Research, N.Y. State Dept. Health, Albany.

It has been demonstrated that poliovirus Type I and Coxsackie virus, Group B, Type 5 occur as crystals in FL amnion cells at a late stage of infection. The crystals were seen in the cytoplasm only. The crystalline pattern resembled that seen in crystals obtained from purified preparations of the viruses. Only the infected cells showed a significant increase in ergastoplasm, the particles of which were similar in size and shape to the poliovirus particles in the crystals. In cell sections containing crystals there was a noticeable decrease in membrane-associated granules. The number of particles formed within a single cell, as estimated from the sections, supports a previously published figure of the order of 10^5 particles per cell.

31 *Immunology*

ADAPTATION OF TWO STRAINS OF RINDERPEST VIRUS TO TISSUE CULTURE. C. J. De Boer. U.S.D.A., A.R.S., A.D.&P.R. Div., Plum Island Animal Disease Lab., Greenport, L. I., New York.

The Pak Chong and Pendik strains of rinderpest virus originating in Thailand and Turkey, respectively, have been adapted to tissue cultures prepared from bovine kidney cells. Freshly prepared cells were infected with infectious bovine spleen suspensions and left to form monolayers. Slight cytopathic changes were observed 18 to 21 days after infection. Formation of clusters of multinucleate cells on the periphery of gaps in and on the edge of monolayers occurred. Gradually, entire sections of the monolayer changed into a mesh-like structure containing increasing numbers of large multinucleate cells or syncytia. In the first subculture infected with inoculum from the 21-day-old original passage, slight cytopathic changes developed in 14 to 15 days. Inoculum for subsequent passages was taken at the peak of the cytopathic changes. Continuous passage resulted in gradual adaptation of both strains with cytopathic changes occurring in 7 days. Following 8 or 9 passages, both strains of the virus had become adapted sufficiently to form a mesh-like structure before confluent monolayers developed. The infectious tissue culture medium is active as a complement-fixing antigen and is capable of producing the disease in cattle. Comparative data on titration in tissue culture and in cattle as well as some immunological aspects are presented.

32 *Immunology*

SUBCLINICAL JAPANESE ENCEPHALITIS IN MAN. Scott B. Halstead and Carl R. Grosz (intr. by E. L. Buescher.) 406th Medical General Laboratory, U. S. Army, Japan.

Assignment of American military personnel to the Far East annually exposes a large group of susceptibles to primary Japanese encephalitis (JE) infection. Three months following the occurrence of 3 cases of overt JE at an air base in Korea, 300 men assigned to the base through the summer were studied for serologic evidence of recent infection to determine: a) the rate of infection; b) the symptoms occurring in subclinical infection; c) the occupational factors influencing exposure and d) the ratio of subclinical to clinical infections. Evidence for recent infection with JE virus was obtained in 9.3% of the test group. Six of the 28 persons with subclinical infection recalled mild illnesses with or without headache and other systemic complaints during the encephalitis season. Most of the infected group had outdoor assignments which exposed them to vectors during the early evening. From this study it was calculated that the ratio of subclinical to overt infection during this outbreak was 25 to 1.

33 *Immunology*

DIFFERENTIAL INACTIVATION OF B-VIRUS SUSPENSIONS OF LIVE POLIOVIRUS. C. W. Hiatt (intr. by R. Murray.) Division of Biologics Standards, National Institutes of Health, Bethesda, Md.

In the presence of trace quantities of toluidine blue (1 to 10 ppm) many animal viruses, including Sabin's B-virus, become sensitized for photodynamic inactivation upon subsequent exposure to visible light. Poliomyelitis and some other enteroviruses are highly resistant to this treatment. Thus B-virus added to monkey kidney tissue culture fluids containing poliovirus can be inactivated with no significant loss in infective titer of the poliovirus. After irradiation, the residual toluidine blue is readily removed by cation exchange resins.

Continuous-flow irradiation equipment has been constructed with a 1000-watt tubular incandescent lamp as a light source. Calibration experiments, using $T2r^+$ coliphage as an actinometer, indicate that this equipment is applicable to the treatment of relatively large volumes of liquid. The process may find practical application as an added safeguard in the manufacture of live attenuated poliovirus vaccine.

34 *Immunology*

INCREASED SUSCEPTIBILITY OF NEWBORN MICE TO INFECTION WITH POLIO AND LCM VIRUSES AFTER PRETREATMENT WITH ADULT MOUSE LYMPHOID TISSUE. Alain Paraf*, R.E. Billingham & Hilary Koprowski. Wistar Inst. of Anatomy & Biology, Phila., Pa.

Mortality in newborn mice is lower than in adult mice following intracerebral inoculation of either Type II polio or LCM virus. Also, the incubation period is much shorter in adult than in newborn mice. Lymphnodes were removed aseptically from adult mice of the inbred C57 strain and processed to obtain suspensions of intact living cells. Fifteen million cells were injected intravenously into newborns of this strain and these baby mice were infected 1-2 days later by the intracerebral route with the respective viruses. The mortality ratios of the pre-injected newborn mice and the virus-injected normal mice were similar. However, the mortality of the pretreated newborn mice was significantly higher than in the non-treated newborn mice. The incubation periods in the pretreated newborn mice and in the adult mice were equally short. No effect was observed when non-living preparations of lymphnodes or serum of adult mice were used instead of intact lymphoid cells. These findings are interpreted in terms of a hypothesis that some diseases are the outcome of autosensitizations in which virus plays a non-specific role as an immunological trigger mechanism. (Supported by an award from the Nat.Acad.of Sciences & National Institutes of Health Grant #2G-142.)

35 *Immunology*

TISSUE CULTURE OF MAMMALIAN MACROPHAGES AND THEIR SUSCEPTIBILITY TO MOUSE HEPATITIS VIRUS. A. Warwick* and F. B. Bang. Johns Hopkins University School of Hygiene and Public Health, Baltimore, Md.

The presence of masses of viruses in the endothelium of the liver of mice infected with mouse hepatitis (electron microscopy) led to a study of the susceptibility of macrophages obtained from mouse liver. These are destroyed within one to two days when mouse liver is cultivated on a collagen substrate. Virus yield from these cultures ranges from 10^{-3} to 10^{-5}. Human embryonic liver also yielded large numbers of macrophages which lasted for more than 3 weeks when grown in a medium containing 50% horse serum. Mouse hepatitis (Nelson strain) is highly selective in its lethal effects for the Princeton strain of mice, and does not damage other strains. The susceptibility of macrophages obtained from different strains of mice is under study, because of large variation in susceptibility of the macrophages when obtained from different sources. Macrophages obtained from different organs of the Princeton mice have been uniformly susceptible when grown with collagen.

251. VIRUSES—Enteroviruses

1 *Biochemistry*

THE NATURE OF THE NON-INFECTIOUS PARTICLES PRODUCED BY POLIOVIRUS INFECTED TISSUE CULTURES TREATED WITH PROFLAVINE. F.L. Schaffer (intr. by C.E. Schwerdt). Virus Laboratory, University of California, Berkeley, California.

Proflavine inhibits production of infectious poliovirus in tissue culture while cytopathic changes occur and non-infectious particles are produced (Ledinko, Virology 6, 512 (1958). This study further elucidates the nature of particles in preparations of low infectivity from cells infected with poliovirus in the presence of proflavine at 5 μg/ml. They were purified by the same procedures employed with infectious virus. Upon ultracentrifugation in a glycerol density gradient, a greenish fluorescent light scattering band was observed in the region ordinarily occupied by infectious virus (fraction D). Lighter particles (fraction C) which contain little or no RNA did not fluoresce. Normal poliovirus particles did not bind proflavine in vitro. Added infectious virus was not separable from dye bound particles by density gradient sedimentation. The ultraviolet absorption spectrum of proflavine-virus was comparable to that of infectious virus. The fluorescence spectrum was similar to that of free proflavine. It is concluded that during synthesis, proflavine (or a derivative thereof) is incorporated, presumably bound to the RNA portion, in particles very similar to infectious poliovirus particles.

XUM

2 *Immunology*

PARALYTIC POLIOMYELITIS IN VACCINATED CHILDREN, Donald M. McLean, Hospital for Sick Children, Toronto, Canada.

During 1959, of 22 children hospitalized with paralytic poliomyelitis, 3 received three or four doses of inactivated (Salk) vaccine, 5 received one or two doses, and 14 were not vaccinated. Faeces of all patients yielded poliovirus (20 were type I, one type II and one type III). Rising homotypic antibody titers were demonstrated in 13 patients (12 type I and one type II), including the 3 type I patients who received full vaccination comprising 3 or 4 doses. Low type 1 antibody levels in acute phase sera which rose during convalescence in fully vaccinated patients suggested lack of response to vaccination. In 1958, of 6 patients with paralytic poliomyelitis who were studied, 2 who excreted poliovirus type I received 3 doses of Salk vaccine 6 months before onset of paralysis. Rising type I antibody levels in both patients and absence of type II and type III antibodies in one patient suggested failure of vaccine to stimulate antibody production. Despite these five vaccination "breakthroughs", the overall efficiency of three doses of Salk vaccine in prevention of paralytic poliomyelitis in Ontario during 1959 was 90%.

3 *Immunology*

MECHANISM OF INTERFERENCE BETWEEN CERTAIN ENTEROVIRUSES IN TISSUE CULTURE. G.D. Hsiung. Yale Univ. School of Med., New Haven, Conn.

In the study of interference between certain enteroviruses it was found that ECHO virus types 1, 5, 6, 9 and 11 were capable of interfering with the production of polioviruses in certain tissue culture systems. Experiments were undertaken to elucidate the mechanism of this interference, which was never more than partial. Kidney cell cultures derived from different animal species were used and virus assays were made in bottle cultures by the plaque technique. When the interfering capacity of certain ECHO viruses was tested in cultures of differing susceptibility, it was noted that interference occurred only in systems which were highly susceptible to the interfering agent. For example, reduction of poliovirus growth by prior infection with ECHO-11 (WB strain) virus was demonstrated only in human cell cultures which were highly sensitive to WB virus, but not in rhesus or patas cells, which were partially susceptible and resistant to this virus. Yields of poliovirus from a single growth cycle showed progressive suppression as the intervals between the introduction of ECHO and poliovirus were increased from 0 to 4 hours. Prior infection with ECHO virus did not reduce the number of cells yielding poliovirus even though the total yield of poliovirus was suppressed.

4 *Immunology*

TRANSMISSION OF POLIOVIRUSES. P. F. Wehrle, Felicitas Hagen* and O. Carbonaro*. State University of N. Y. Upstate Med. Center, Syracuse, N. Y.

An outbreak of poliovirus type I infection, including two cases of paralytic poliomyelitis, occurred in 1958 among children attending a recreational summer school program in central New York. The following year six children in the same school were inoculated with attenuated Leon 12 a;b (Sabin) strain of poliovirus type III. Sera and frequent throat and fecal samples were obtained from the approximately 100 school children and siblings during similar periods of both years. The spread of the natural type I infection during 1958 was extensive, and ultimately involved nearly half of the children attending the school. In contrast, the attenuated type III infection, although successfully initiated, had very limited spread both in the inoculated families and to other children in the school studied. Data regarding pharyngeal infection and antibody levels will be presented. (Supported by a grant from the National Institutes of Health).

5 *Immunology*

CHARACTERISTICS OF ECHO 4 (SHROPSHIRE) VIRUS ISOLATED DURING EPIDEMIC OF ASEPTIC MENINGITIS. Almen L. Barron* and David T. Karzon. Depts. of Bacteriology and Immunology and Pediatrics, Univ. of Buffalo School of Medicine.

A serologic variant (prototype Shropshire) of ECHO 4 virus was the predominant isolate from an epidemic of aseptic meningitis in Buffalo, N.Y., 1956. The antigenic relationship of the Shropshire-like strains and other ECHO 4 viruses was studied in monkey kidney tissue cultures using tube neutralization and plaque reduction tests. Using rabbit antisera, tube tests indicated that Buffalo epidemic strains were antigenically homogeneous (titer ratio, R x 100 = 112) and distinct from the prototype ECHO 4, Pesascek (R x 100 = 3-12). ECHO 4 viruses examined from other outbreaks were like Pesascek. The difference between Shropshire and Pesascek groups was quantitatively magnified by plaque reduction method. However, when monkey antisera were used antigenic differences noted with rabbit antisera were less apparent. Although tube tests using either Shropshire or Pesascek virus showed little or no antibody rise with Buffalo epidemic patient sera, satisfactory rises were obtained with both viruses by plaque reduction method. Two South African strains, related to Pesascek, were found to be unusually good antigens in tube tests for detection of neutralizing antibody in rabbit and monkey sera and could be used to demonstrate antibody rises in human sera.

6 *Immunology*

CHARACTERISTICS AND CHEMOPROPHYLAXIS OF ECHO-9' VIRUS INFECTION IN MICE. Kenneth W. Cochran and John T. Headington*, Depts. of Epidemiology and Pathology, Univ. of Mich., Ann Arbor.

Certain enteroviruses have been found to vary in their susceptibility to the antiviral action of certain substances in vitro. Several strains of ECHO-9 isolated from the 1958 Detroit "poliomyelitis" epidemic were found pathogenic for suckling mice and gerbils and offered the possibility of studying some of these actions in vivo. "Holt" strain appeared more virulent and was selected for further mouse-to-mouse passage. Suspensions of eviscerated carcasses of very young mice or the hind leg muscles of older mice produced paralysis when injected IP. In agreement with this and in common with Coxsackie A viruses, microsconic lesions were seen in skeletal muscle. Lesions were not seen in other organs, and suspensions of brain and lungs failed to paralyze newborn mice. In the first 8 mouse passages the time of onset of paralysis decreased from 5-7 days to 2-3 and the maximum age of susceptibility increased to 18-19 days. Adult mice were insusceptible when inoculated IP, IC, or IS, and monkeys IC. Also in common with Coxsackie-A viruses, ECHO-9' (Holt) increased the pathogenicity of MEF_1 poliovirus in mice. Helenine and MS-8450, active in vivo against poliovirus, protected mice against ECHO-9' (Holt) whereas diazouracil, polylysine-20, and Maclura pomifera, also active against MEF_1 poliovirus in mice, had essentially no effect.

7 *Immunology*

EFFECTS OF CHRONIC WHOLE-BODY X-RADIATION ON ANTIBODY RESPONSES TO AQUEOUS AND EMULSIFIED INFLUENZA VIRUS VACCINES. Byron S. Berlin (introduced by Thomas Francis Jr.) Virus Laboratory, Dept. of Epidemiology, School of Public Health, Univ. of Mich., Ann Arbor, Michigan.

Effects of chronic x-irradiation on responses to emulsified vaccines have not previously been defined. To compare effects of such irradiation on antibody responses to aqueous and emulsified influenza virus vaccines, mice were exposed to X-ray for various intervals beginning one day before vaccination. Depending on the daily dose and the number of daily exposures, slight enhancement or depression of antibody levels was seen. With equal exposure, responses to emulsified vaccine were depressed more than those to aqueous vaccine. The finding that irradiation begun one week after vaccination altered responses to emulsified vaccine more than responses to aqueous vaccine confirms the hypothesis that antigenic stimulation by emulsified vaccine is more protracted than that by aqueous vaccine. Effects of equivalent cumulative doses of X-ray were less when given over an interval of more than one week than when given over a shorter interval. This finding suggests active repair of X-ray injury can take place even during continued exposure.

(This investigation was conducted under the auspices of the Commission on Influenza, AFEB, and was supported by the Office of the Surgeon General, U. S. Army, Washington, D. C.)

8 *Immunology*

SUSCEPTIBILITY AND RESISTANCE OF CELLS IN VITRO TO INFECTION BY COXSACKIE A-9 VIRUS. L. C. McLaren and J. J. Holland (Introduced by J. T. Syverton). Dept. Bacteriology, Univ. Minnesota, Minneapolis.

Multiple human cell types in primary culture proved susceptible to infection by types 1-3 polioviruses, Coxsackie B1-5 and Coxsackie A-9 virus. Contrariwise, human cell lines in continuous culture derived from both normal and malignant tissues proved resistant to Coxsackie A-9 but not to the other enteroviruses. Infection of resistant cells by ribonucleic acid prepared from Coxsackie A-9 indicate that these cells were physiologically capable of synthesizing fully infectious A-9 virus. Human amnion and kidney cells derived from primary cultures proved resistant when in continuous culture. Resistant cell strains, or debris prepared therefrom, failed to adsorb Coxsackie A-9 virus. These findings indicate for cells in continuous culture loss of receptors specific for Coxsackie A-9 virus. Comparative findings for the kinetics of adsorption of Coxsackie A-9 virus and other enteroviruses by susceptible cells will be presented. Enhancement of adsorption by Ca and Mg ions was found limited to Coxsackie A-9 virus infection.

9 *Immunology*

SELECTIVE INHIBITION OF ENTEROVIRUSES BY 2-(α-HYDROXYBENZYL)-BENZIMIDAZOLE (HBB). Hans J. Eggers* and Igor Tamm. Rockefeller Institute, New York.

Earlier work showed that 2-(α-hdyroxybenzyl)-benzimidazole (HBB) inhibits multiplication and cytopathic effects of polio type 2 but not of influenza B virus. In further studies monkey kidney tissue cultures were inoculated with 100-500 $TCID_{50}$ of various viruses and development of cell damage in untreated cultures was compared to that in cultures held in the presence of HBB at 22 or 110 μg/ml. A virus was considered to be inhibited by HBB if more than 75% reduction in cell damage was observed in the presence of 22 μg/ml. Inhibition was considered slight if more than 75% protection was observed in the presence of 110 but not 22 μg/ml. Inhibited were the following viruses: Coxsackie A9,B2,B3,B5 and ECHO prototypes 4,6,7,9,11,19. Slightly inhibited were polio 1 (Mahoney) and polio 3 (Saukett). Not inhibited were the following: mumps, croup-associated virus, hemadsorption 1, adeno 2, arbor B (West Nile) and C (Oriboca, Marituba), vaccinia, reovirus prototypes, Coxsackie A7, A16 and ECHO 23. At 22 μg/ml HBB caused no microscopic changes in cells; at 110 μg/ml minor changes developed. At these concentrations HBB had no effect on incorporation of adenosine-8-C^{14} into RNA, on incorporation of C^{14}-L-alanine into proteins, or on oxygen uptake of monkey kidney cells. Among synthetic chemical compounds HBB is uniquely selective in its virus inhibitory action.

10 *Immunology*

COMPARATIVE SUSCEPTIBILITY OF HAMSTERS AND MICE TO COXSACKIE B-3 VIRUS. Theodore Burnstein (intr. by M. Michael Sigel.) Variety Children's Research Foundation, Miami, Florida.

Mice and hamsters are considered to be the animals of choice for studies on Coxsackie viruses. While these viruses have been studied extensively in mice, their effects on hamsters have received relatively little attention. The present report compares the response of these two species to Coxsackie B-3 virus as influenced by age. Concurrent tests employing stock virus were made in suckling hamsters and mice of varying ages. Criteria studied were titers of virus and susceptibility to lethal effects of virus. It was found that at a given age virus titers were consistently higher in hamsters than in mice and that the period of susceptibility of hamsters was about twice that of mice. These data suggest that greater use be made of hamsters for the isolation of certain enteroviruses.

11 *Immunology*

VIRAL STUDIES OF AN OUTBREAK OF CHOLERA. Andrew Abraham (intr. by F. S. Cheever.) Dept. of Microbiology, School of Medicine, University of Pittsburgh.

From only one of 40 stool specimen obtained from an outbreak of cholera in Thailand a virus was isolated in human amnion (FL line) cell cultures. It was not neutralized by Polio (1-3), Coxsackie (A 1-19, B 1-6) or ECHO (1-26) immunsera; it produced enterovirus-like CPE in human cell cultures only, formed uniform, small, clear plaques, was ether resistant, produced paralysis of the extremities in suckling mice with severe degenerative changes in the muscles, and, with the exception of a small burst size, exhibited human enterovirus-like growth characteristics. Antibodies were not found in the patient's acute or convalescent serum. Of the 23 pairs of sera tested only one showed the presence of antibodies in low titer. No antibody was detected in gamma globulin or adult sera of American origin. A rectal swab from a child in Pittsburgh with transient diarrhoea also yielded a somewhat similiar virus only in FL cultures. This was characterized by a lower titer and later appearance of CPE, a lack of pathogenicity for suckling mice, and formation of tiny, cloudy plaques on FL monolayer cultures. Preliminary serological studies suggest that these two viruses and the COE virus represent three immunologically unrelated agents. (Supported by a grant from the Office of the Surgeon General, U. S. Army.)

12 *Immunology*

CORRELATIONS OF IN VITRO MARKERS OF POLIOVIRUS STRAINS AND MONKEY NEUROVIRULENCE. I. COMPARISON OF VACCINE AND FIELD STRAINS. M. Benyesh-Melnick* and J. L. Melnick. Dept. Virology & Epidemiology, Baylor Univ. Col. of Med., Houston, Texas.

Virulent strains of poliovirus recovered from paralytic patients usually possess the d+ (growth under agar at low bicarbonate concentration) and t+ (growth at 40°C) markers, in contrast to the d-t- markers of the live poliovaccine strains. Over 100 strains recovered from vaccinated children have been analyzed for these markers. While the majority of the progeny strains retained the markers of the vaccine virus, an appreciable number changed from d- to d+, and from t- to t+. While the vaccine strains were negative after intracerebral inoculation of monkeys with 10^7 TCD_{50} of virus, and as much as 10^4 TCD_{50} were required to bring down 50% of monkeys inoculated intraspinally, d+t+ strains produced paralysis after intracerebral inoculation, and as little as 10 TCD_{50} or less brought down monkeys with paralytic poliomyelitis after intraspinal inoculation. Two d+t+ strains were also tested in chimpanzees, and both produced paralysis, in contrast to the avirulence exhibited by the d-t- vaccines in this primate.

13 *Immunology*

CORRELATIONS OF IN VITRO MARKERS OF POLIOVIRUS STRAINS AND MONKEY NEUROVIRULENCE. II. INTRAMUSCULAR INOCULATION OF VACCINE PROGENY STRAINS, AND SERIAL SPINAL CORD PASSAGE OF t- STRAINS. J. L. Melnick and M. Benyesh-Melnick*. Dept. Virology & Epidemiology, Baylor Univ. Col. of Med., Houston, Texas.

To avoid the trauma associated with direct inoculation of virus into the simian central nervous system, especially by the intraspinal route, a number of strains recovered from children infected with live poliovaccine were studied in monkeys by the intramuscular route. Again it was found that strains which showed reversions in the d and t markers also showed increased monkey neurovirulence. Certain strains which went from d-t- to d+t+ were found to produce paralysis after inoculation of less than 100 TCD_{50}. Using 10% suspensions of spinal cord as inoculum, paralytogenic virus was passed through 4 serial monkey transfers. The virus recovered from the CNS in all 4 transfers retained the t- marker. The results indicate (1) that possession of the t- marker by itself is not sufficient to indicate that a poliovirus is highly attenuated and (2) that an attenuated t- vaccine strain may retain its t- marker after multiplication in man and yet show an increase in monkey neurovirulence.

XUM

14 *Immunology*

MULTIPLICATION OF POLIOVIRUS IN CELLS PHOTOSENSITIZED BY NEUTRAL RED. Derek Crowther (intr. by J. L. Melnick.) Dept. Virology & Epidemiology, Baylor Univ. Col. of Med., Houston, Texas.

A depression of the plaque count of poliovirus has been reported in cell monolayers containing neutral red and exposed to light. Growth studies of type 1 polioviruses were undertaken in previously photosensitized monkey kidney cells. It was found that cells sensitized with 1/240,000 neutral red and then exposed for one hour to white light from a fluorescent lamp appeared normal for at least 3 days, but such conditioned cells produced only small amounts of virus. Growth curves of virulent Mahoney and attenuated LSc in cells conditioned in this way showed an average depression, throughout an 8-hour growth cycle, of approximately 96% for both viruses. As the viruses adsorbed equally well onto conditioned and unconditioned cells, the block in cells conditioned before application of the virus occurred in the intracellular development of infective virus.

15 *Immunology*

EFFECTS OF NEUTRAL RED AND LIGHT ON POLIOVIRUSES. Edward M. Opton* and Robert H. Green. Yale University School of Medicine, New Haven, Connecticut.

Neutral red, the dye often used in viral plaque assays, has been shown to depress the plaque counts of some viruses. Recently the photosensitizing effect of neutral red on agar-overlaid tissue cultures has been recognized. It is not known, however, whether diminutions of plaque titers in the presence of neutral red were direct effects upon the virus, or were mediated by photosensitization of the host cells. To elucidate this question suspensions of Mahoney and LSc strains of poliovirus type I were mixed with neutral red and exposed to light; appropriate dilutions of the virus suspensions were then made and plaque assays performed in the usual manner in bottle cultures of rhesus monkey kidney cells. It was found that the combined exposure of these virus strains to light and neutral red resulted in slight but consistent enhancement of titer of the LSc strain and reduction in titer of the Mahoney strain. It is concluded that the response in each instance is a strain characteristic. (Supported by a grant from the National Foundation.)

252. VIRUSES—Myxoviruses

1 *Biochemistry*

ISOLATION OF NEURAMINIDASE FROM INFLUENZA VIRUS – Lewis W. Mayron* and M.E. Rafelson, Jr. Univ. of Ill., College of Med., Chicago, Ill.

Treatment of PR8 and Asian virus with trypsin at pH 7 for 16 hr. at 37°, followed by dialysis (to remove autolyzed trypsin) and ultracentrifugation, yielded an enzymatically active fraction containing 70-90% of the neuraminidase activity originally present in the virus. The enzyme activity remained in the supernatant solution after centrifugation at 105,000 g. for 3 hr. and passed through filters with 50 mμ pores. Electron micrographs revealed no intact virus particles. The product liberated by enzyme action on oroscmucoid, a milk protein and neuramin-lactose was identified as N-acetylneuraminic acid (NANA). Untreated virus preparations and appropriate controls showed no enzyme activity under the above conditions. No trypsin activity was demonstrable in the enzyme preparation by the azo-albumin technique. In addition trypsin does not liberate NANA from the substrates used. The enzyme neither agglutinates erythrocytes nor blocks their agglutination by an added dose of indicator virus. At 37° the enzyme destroys the receptor sites on erythrocytes for Lee indicator virus.

2 *Pathology*

THERMORESISTANT INFLUENZA A VIRUS. Robert A. Siem*, Henry Ly*, David T. Imagawa and John M. Adams. Depts. of Pediatrics and Infectious Diseases, School of Medicine, Univ. of Calif., Los Angeles, and Veterans Administration Hosp., Long Beach, Calif.

Smorodintsev demonstrated greater persistence for a thermoresistant strain of influenza virus as compared to stock virus when inoculated into animals. Our studies with PR8 virus show that an apparent selective mechanism is involved since heat resistant strains retain this property upon repeated serial passage. Comparison of passages at low and high (clone) dilutions shows reversion to original heat susceptibility at low dilutions, whereas at limiting dilutions heat resistance is retained. In comparing the resistant strain to original stock virus, no differences were apparent in initial HA titers or in the production of influenzal toxin from 11 day embryonated eggs. Antigenic differences were not apparent in mice as demonstrated by HI titers and by cross protection studies. Infectivity for both embryonated eggs and mice was from 1.0 to 2.0 logs higher for the original stock virus. Smorodintsev showed that heat inactivated virus rapidly loses its immunogenic properties in vivo from the action of normal tissue enzymes. We have extended this study to determine persistence of antigenicity in mice as determined by HI titers and anamnestic responses.

3 *Pathology*

CYTOPATHOLOGICAL AND CYTOCHEMICAL OBSERVATIONS OF RNA SYNTHESIS IN HELA AND MK CELLS INFECTED WITH PARAINFLUENZA VIRUS. R. Gerald Suskind* and Robert Love. Natl. Cancer Institute, Bethesda, Md.

The cytopathologic changes of parainfluenza virus infection vary with the host cell type (Annals, N. Y. Acad. Sciences 81: 1, 62, 1959). A symbiosis of virus and host cell without necrosis is observed in MK, whereas syncytium formation and cell death occurs in Hela. Concomitant differences in the sites of viral RNA synthesis were found in the two systems with the toluidine blue-molybdate technique (J. Histochem. and Cytochem. 7: 164, 1959). In Hela an increase in RNA is seen two hours after infection with 10^3 and 10^7 T. C. doses of type 1 virus, in the usual sites of cellular RNA synthesis, the nucleolus and parachromatin associated with syncytium formation. This is followed rapidly either by extrusion of the RNA or by nuclear degeneration and persistence of nuclear fragments in the cytoplasm of the syncytium. Mitosis is not observed. In MK, the sites of cellular RNA synthesis are unaltered; mitosis is vigorous in infected cells. Phase dense RNP bodies appear in the cytoplasm within 10-16 hours after infection. Similar bodies are seen in the nucleoplasm within 48 hours with or without cytoplasmic RNP bodies. The relationship to viral synthesis is discussed.

4 *Immunology*

VIRAL INTERFERENCE AT A DISTANT SITE? Sidney E. Grossberg*, Edward W. Hook*, and Robert R. Wagner. The Johns Hopkins, Minnesota and Cornell Medical Schools, Baltimore, Minnespolis and New York.

Cerebral hemorrhages and death of chicken embryos caused by I.V. injection of neurotropic (NWS) influenza virus can be prevented by prior allantoic infection with WS influenza or other myxoviruses. Multiplication of NWS virus in brains of resistant embryos was reduced appreciably. Protection against cerebral hemorrhages was afforded by fully infectious WS virus and not be virus inactivated by UV light or heat. Moreover, resistance did not depend on multiplicity of interfering virus, but did require that active allantoic infection be established 8-16 hours prior to I.V. challenge. The small quantity of interfering WS virus disseminated from allantoic sac to blood and brain was deemed insufficient to account for resistance to I.V. challenge with NWS virus. No protection was noted after I.V. injection of suspensions of WS virus. However, I.V. or allantoic injection of allantoic fluid, freed of infectious WS virus by centrifugation and heat, protected embryos against action of I.V. NWS virus. The resistance-promoting factor produced in vivo by infected allantoic cells of chicken embryos has many of the properties of interferon.

5 *Immunology*

BIOPHYSICAL AND SEROLOGICAL PROPERTIES OF INFLUENZA VIRUS AND RELATED HOST MATERIALS. L.H.Fromhagen* and E.H.Lennette, California State Dept.Public Health, Berkeley, Calif.

The properties of five strains of influenza virus grown in ecbryonated eggs and of chorioallantoic material (CAM), were investigated during a continuing study of the structure of influenza virus. Purified preparations of older, highly passaged strains, PR8/34 and Lee/41 were found to possess an infectious, hemagglutinating component whose electrophoretic mobility, electron microscopic appearance, and reactivity to anti-CAM sera are identical to CAM. The newer, less passaged strains, Spirup/48, Denver/57, and Asian/57 consist, on the other hand, of several infectious electrophoretic components with mobilities fractional to those of CAM and the older influenza strains, lower reactivities to anti-CAM sera, and a consistent electron microscopic appearance. Nevertheless, both the newer and older strains yield, upon ether treatment, group(S) and strain (V) antigens which are equally related, serologically and biophysically, to CAM ether fractions. Large, bag-like particles (100-300 mμ), elevated intrinsic viscosities, and low sedimentation rates, characteristic of some CAM preparations have also been noted in preparations of 'incomplete' PR8 virus. These data are consistent with the thesis that CAM constitutes the major portion of both the external and internal aspects of the influenza virus particle.

6 *Immunology*

CERTAIN BIOLOGICAL CHARACTERISTICS OF MYXOVIRUS PARA-INFLUENZA 3. F. W. Denny, Jr. Western Reserve University School of Medicine, Cleveland, Ohio.

Myxovirus para-influenza 3 is one cause of respiratory tract infections (Chanock et al., JAMA 169, 548, 1959). Studies were done to determine characteristics of its growth, stability, and reaction with specific antibody. HeLa cells were used throughout and viral growth was determined by cytopathic effect. The latent period of the initial multiplication cycle was 15-18 hours; the incremental period of growth was 5 hours. Infectious virus was released promptly from cells. The virus was stable for 30 min. from pH 4.5 to pH 9.5 and at temperatures from 40-45° C; 50° C for 30 min. reduced the infectivity titer 90-99%; 56° C almost completely inactivated the virus in 10-20 min. Storage at 22° and 36° for 6-12 hours resulted in no reduction of infectivity titer; subsequently, titers gradually decreased until by 7 days 90-99% of virus was inactivated. There was no detectable loss of virus at 4° C for 7 days or at -25° C for 60 days. Quantitative neutralization tests showed that the slope of the neutralization line was approximately 2. These data indicate that the virus grows well, is stable and may be neutralized quantitatively.

7 *Immunology*

THE GENETIC DETERMINATION OF INFLUENZA VIRAL MORPHOLOGY. Edwin D. Kilbourne and James S. Murphy*. Cornell University Medical College and Rockefeller Institute, New York, N. Y.

Inoculation of the allantoic sac of the chick embryo with newly isolated, filamentous Asian strains of influenza virus after the injection of thermally-inactivated, completely non-infective PR8 virus results in the emergence and predominance, within 2-3 passages, of virus of Asian antigenicity but PR8-like growth capacity and spherical morphology. Concomitant control passages of either parent virus alone reaffirm the inability of the inactivated PR8 virus to multiply, and the failure of the infective Asian virus to change from a predominantly filamentous morphology observed on electron microscopy. By the use of horse serum inhibitor effective in total neutralization of the infective parent, isolation and quantitation of recombinant progeny is possible. With this technique reciprocal recombinants of reduced growth rate, PR8 antigenicity and filamentous morphology have been isolated. As back mutation from spherical to filamentous form is not known to occur spontaneously, these experiments strongly suggest that the morphologic expression of an influenza virus is a transferable and heritable factor.

8 *Immunology*

VARIATION IN SUSCEPTIBILITY TO THE INDUCTION OF VIRAL INTERFERENCE WITH VARIATION IN HOST AGE. K. Marilyn Smart* and Edwin D. Kilbourne. Cornell University Medical College, New York.

The introduction of inactivated influenza viruses into in vitro cultures of chick embryo chorioallantoic membranes induces interference with the multiplication of infective virus inoculated subsequently. Under conditions which result in equivalent final yields of virus from membranes of differing age, it has been shown that the minimal dose of virus required for the induction of interference varies inversely with membrane age in the range of 8-13 days. Thus, more virus particles are required to induce interference in younger embryos, despite the lesser numbers of cells in such membranes. The preventability of interference with cortisone is similarly age-related; interference in membranes of 10 days or older is inhibited with cortisone, but the lesser interference induced in younger membranes is not. It is probable that these differing effects of inactivated virus are reciprocally related to the higher metabolic and viral synthetic activity of younger embryos. Preliminary data suggests a possible relation to membrane glucose utilization.

9 *Immunology*

INTERACTION OF MYXOVIRUSES WITH HELA CELLS AS REVEALED BY FLUORESCENT ANTIBODY. E. Frederick Wheelock (intr. by Igor Tamm.) Rockefeller Institute, New York.

Interaction of Newcastle disease and influenza A viruses with HeLa cells was studied quantitatively by fluorescent antibody technique using complete monolayers of cells on 11x38 mm coverslips. Determinations of infective units of NDV were based on enumeration of specifically fluorescent cells after one cycle of multiplication. The number of fluorescent cells was directly proportional to virus concentration. Time of first appearance and amount of antigen per cell during the incremental period were dependent on multiplicity. The critical point following infection after which infected cells no longer can undergo mitosis and divide was also dependent on multiplicity. Before the critical point, mitosis occurred in cells which had produced and released infective virus. After simultaneous inoculation of cultures with NDV and influenza virus, many cells contained antigens of both viruses and numerous doubly infected multinucleated giant cells were seen. In interference experiments a large inoculum of NDV infected all cells within 30 seconds. Such was not the case with influenza virus. The interval between interfering and challenge viruses was varied and the inability of challenge virus antigen to develop in cells infected with interfering virus was considered to indicate interference. NDV required much less time to establish interference against influenza than influenza against NDV.

10 *Immunology*

A STUDY IN VITRO OF COMPONENTS IN THE TRANSMISSION CYCLE OF SWINE INFLUENZA VIRUS. W.D.Peterson, Jr.* (Introduced by F.M. Davenport) Dept. of Epidemiology, School of Public Health, Ann Arbor, Michigan.

Swine lungs, swine lungworms, and earthworms, each a component in the transmission cycle of swine influenza virus (Shope, R.E., J. Exper. Med., 74:49, 1941), were grown or maintained in vitro. Swine influenza virus was added to each preparation, and the fate of virus followed. Virus multiplied readily in cultures of lung. Virus was adsorbed to lungworms, and could be recovered for prolonged periods of time. However, no increase of virus in lungworms or medium was detected. No multiplication or prolonged survival of virus was observed in whole earthworms, or in cultures of minced earthworms. Inhibitors of viral hemagglutination were found in extracts of lung, bronchial secretions, serum, and lungworms, but not in extracts of earthworms. The properties of these inhibitors differed, as shown by treatment with heat, KIO_4, or active virus. The demonstration that virus adsorbs to lungworms, and that receptor like substances are present in lungworm extracts might be considered support for the thesis that lungworms play an active role in the transmission of swine influenza virus. Contrariwise, interaction between virus and earthworms was not observed.

11 *Immunology*

THE ADVANTAGES OF MINERAL OIL EMULSIFIED INFLUENZA VIRUS VACCINES WHEN USED IN A TWO DOSE SCHEDULE OF INOCULATIONS. A.V. Hennessy and F.M.Davenport, Dept. of Epidemiology, School of Public Health, University of Michigan, Ann Arbor, Michigan.

Failure to obtain regularly optimal levels of antibody in antigenically inexperienced persons by using two doses of aqueous influenza virus vaccines led to a comparative study of antibody responses in groups of children and adults given two doses of polyvalent aqueous or emulsified vaccines or vaccine of either type followed by the other. Antibody and antibody increase were measured by hemagglutination-inhibition. In children, two doses of aqueous vaccine induced higher levels of antibody to swine and PR8 strains than did one dose. In adults the second dose of aqueous vaccine was ineffectual for enhancing Swine, A, and A-prime antibodies. However, in both groups the use of emulsified vaccine for either the first or second dose gave distinctly higher and more uniform antibody levels against all strains. Two doses of emulsified vaccine gave the best results in both age groups. Persistence of high levels of antibody against all strains as measured in sera obtained one year after the second vaccination was clearly favored by the use of emulsified vaccines.

This investigation was conducted under the auspices of the Commission on Influenza, AFEB, and was supported by the Office of the Surgeon General, U. S. Army, Washington, D.C.

12 *Immunology*

ANTIGENIC VARIANTS OF INFLUENZA A VIRUS. VI. RESULTS OF REPEATED PASSAGES STARTING WITH THE SAME VARIANT. Dorothy Hamre and C. G. Loosli. Department of Medicine, U. of Chicago, Chicago, Ill.

In previous experiments seven antigenic variants of influenza A virus (PR8) were isolated from passages of this virus and its succeeding variants in mice given homologous vaccine. In the present study, one series of passages, using the fourth variant of the original line, was repeated four times. Four new variants were obtained, two of which were identical. All of the new variants could be distinguished from the fifth variant isolated in the original passages. However, cross hemagglutinin-inhibition, neutralization and antibody absorption tests demonstrated that all of these variants shared some antigenic components; none contained a completely different set of antigens. (Supported by a grant from the Office of the Surgeon General, Department of the Army)

253. VIRUSES—Phage

1 *Biochemistry*

HEAD STRUCTURE OF BACTERIOPHAGE T2. Lloyd M. Kozloff, Donald J. Cummings,* and Murl Lute.* Dept. of Biochemistry, University of Chicago, Chicago, Ill.

The head of T2 phage can exist in two interconvertible configurations; one form is 15-20 mμ longer than the other. This head configuration depends on both pH and temperature; the conversion to the longer head form is favored at high temperature and pH. The longer head form has a S_{20} of 710 as compared to a S_{20} of 1020 for the shorter form. After formol fixation the head can no longer change shape. The change in head shape is a feature of the head protein rather than the DNA since T2 ghosts exhibit two head shapes and two sedimentation forms (S_{20} = 166 and S_{20} = 256) depending in a similar fashion on pH and temperature. A change in head configuration from the long to the short form occurs during the injection of the viral DNA into the host cell. Formol was used to fix the head form after injection and the emptied phage heads were shaken off the host cell. Although both intact phage and phage ghosts would be expected to be in the long head configuration under the conditions where injection occurred all empty heads were in the short form.

2 *Biochemistry*

AMINO ACID COMPOSITION OF BACTERIOPHAGE COMPONENTS. M. L. Coval*, V. Møller*, and H. Van Vunakis. Grad. Dept. of Biochemistry, Brandeis Univ., Waltham, Mass.

The composition of phages T2, T4 and T6 (active on E. coli B) and P22 (active on S. typhimurium) has been determined using the Spackman, Stein and Moore automatic amino acid analyzer. The ghosts of these phages and the head subunits, tail, internal protein and DNA of T2 have also been analyzed. The results revealed (1) hydrolysis under N2 decreases the breakdown of tyrosine, serine and threonine which is excessive in the presence of DNA; (2) there are several unknown ninhydrin reacting substances present in phage hydrolysates which are not found in ghost or DNA hydrolysates and which are not the polyamines commonly associated with phage; (3) there are differences in composition between phages and between the proteins of a single phage, e.g., the internal protein of T2, with its high content of lysine and histidine and no cystine, differs from any other component of T2; and (4) T2 DNA preparations, purified by a combination of procedures, contain amino acids which do not correspond to the qualitative or quantitative composition of any phage protein. Whether these amino acids represent unspecifically bound impurities or whether they are an integral part of the DNA molecule is under investigation.

3 *Biochemistry*

FORMATION OF T2 PHAGE AND dCMP-HYDROXYMETHYLASE BY PREPARATIONS DERIVED FROM SPHEROPLASTS. K. Ebisuzaki*, E.A.Figueiredo*, S. Schlesinger* and G. R. Greenberg. Dept. of Biological Chemistry, University of Michigan, Ann Arbor.

Urea-inactivated T2 phage (UIP) interacts with E.coli spheroplasts to form phage (Spizizen and Fraser et al.) and dCMP-hydroxymethylase. Spheroplasts of E.coli B/2 were shocked into dilute buffer, frozen and thawed (FT), and after additional freezing and thawing, centrifuged (FT-precipitate). These preparations interact with UIP to form phage in the presence of casein digest, Difco yeast extract, ATP generator, and the supernate of FT-precipitate or a phenol-treated extract of E. coli. With FT a typical yield in 60 min. is 4.7×10^7 phage/10^9 cell-equivalents (100%), minus ATP generator 0.12%, minus UIP or FT $< 10^{-5}$%. FT-precipitate shows a lower yield but greater stimulation by phenol fraction or supernate which appear to have high molecular weights. Addition of albumin and sucrose to these FT preparations increases the phage yield and abolishes the requirements for ATP and phenol fraction. While these appear to be complex particles, the albumin effect is not understood and undamaged spheroplasts are not yet excluded. Phage DNA plus various phage fractions did not replace UIP in initiating enzyme and phage formation. UIP is a complex, DNase-insensitive, trypsin-sensitive particle.

4 *Biochemistry*

BACTERIOPHAGE P1 INDUCTION BY THYMINE DEPRIVATION. N. E. Melechen, Saint Louis Univ. School of Med., St. Louis, Mo., and P. D. Skaar, Montana State College, Bozeman, Mont. (intr. by M. Weber).

The temperate bacteriophage P1b is incapable of completing the lytic cycle in the thymineless E. coli strain B-3 in absence of exogenous thymine. P1b infected B-3 are, however, less subject to death in the absence of thymine than uninfected B-3. The P1b prophage does not overcome the host's thymine requirement as B-3 lysogenic for P1b also undergo thymineless death. In over 20% of these, P1b is induced. Except for very small yields of phage in thymineless cultures (less than 1 per induced bacterium), induced lysogenics, like cells infected with exogenous phage, do not release phage in the absence of thymine. Maximum induction is reached after 90 minutes of thymine starvation. Addition of thymine to samples taken at various intervals during thymine deprivation results in "normal" yields of up to 37 phages per induced bacterium.

Thus, thymine deprivation, presumably through interruption of DNA synthesis, leads to induction of these lysogenic bacteria. (Supported by grants from The National Foundation and National Cancer Institute, respectively.)

5 *Pharmacology*

INACTIVATION OF T2 BACTERIOPHAGE AFTER INCUBATION WITH SYNTHETIC POLYSACCHARIDE DERIVATIVES. B.G.Young and P.T.More (intr. by M. J. Shear.) Natl. Cancer Inst., Bethesda, Md.

Incubation of T2 bacteriophage at pH 4.6 with small amounts of polyglucose sulfuric acid (75 μg/ml.) or polyglucose carboxylic acid (300 μg/ml.) led to an irreversible loss of infectivity for E. coli when assayed at the usual pH. Infectivity was not lost after similar incubation (15 min. 0° C.) with sulfuric or acetic acid. At somewhat lower pH (4.2) these low molecular weight acids also reduced infectivity, but only to a smaller extent; infectivity in these cases was restored when the pH was brought to 7-8 for 15 min. before plating. No such reversion was obtained when inactivation was produced with macroanions. A different type of inactivation was produced on incubation at pH 9.6 with 250 μg/ml. glucose-dialdehyde, or of polyglucose-dialdehyde, prepared by periodate oxidation from glucose or polyglucose. In these cases, there was no difference between the effects produced by low and high molecular weight compounds; moreover, the inactivation was accompanied by release of DNA. The inactivation with aldehydes was more dependent on temperature than was the inactivation with macroanions.

254. VITAMINS

1 *Biochemistry*

EVIDENCE FOR A SINGLE ENZYME REDUCING FOLATE AND DIHYDROFOLATE. Sigmund F. Zakrzewski (intr. by Charles A. Nichol.) Dept. of Experimental Therapeutics, Roswell Park Memorial Institute, Buffalo, N. Y.

Partially purified folic acid reductase from chicken liver reduces dihydrofolate (FH_2) at least twenty times faster than folate (F). In each case tetrahydrofolate is the end product. While all attempts to separate the activity for FH_2 from the activity for F were unsuccessful, evidence has accumulated indicating that both reactions are carried out by the same enzyme. The ratio of the activity for FH_2 and F remains constant during purification. TPNH is required for the reduction of both substrates. DPNH is inactive. Amethopterin inhibits both reactions pseudoirreversibly. Although the turnover number for FH_2 (467 moles/min./mole of enzyme) is much larger than for F (23), the amount of the antagonist just sufficient to inhibit both reactions completely is the same in each case. F inhibits the reduction of FH_2 competitively. The affinity of F for the enzyme was the same when it acted as an inhibitor ($K_I = 8 \times 10^{-6}$ M) of the reduction of FH_2 or as a substrate ($Km = 7.9 \times 10^{-6}$ M). The Km for FH_2 was also similar (8.6×10^{-6} M). Heating inactivates the reduction of F and FH_2 at the same rate. This evidence indicates that a single enzyme can mediate the reduction of both folate and dihydrofolate.

2 *Biochemistry*

SARCOSINE DEHYDROGENASE.
G. P. Mell* and F. M. Huennekens. Dept. of Biochemistry, Univ. of Washington, Seattle, Washington.

From acetone powders of rat liver mitochondria a soluble sarcosine dehydrogenase (cf. also Knowles and Sakami, Federation Proc. 18, 263 (1959)) has been extracted and purified by ammonium sulfate fractionation and negative adsorption on calcium phosphate gel. Two separate ammonium sulfate fractions (33-45% and 45-60%) are required for activity. The dehydrogenation of sarcosine may be linked to the reduction of cytochrome C, 2,6-dichlorophenol indophenol, or phenazine methosulfate (but not methylene blue). The oxidation products are glycine and HCHO. In the presence of serine hydroxymethylase and tetrahydrofolic acid, the end-product of the reaction is L-serine. Neither tetrahydrofolic acid nor dihydrofolic acid is apparently required for the oxidative step. The enzyme system is inhibited by p-chloromercuribenzoate, atebrine, methoxyacetate, and Zn^{++}, but not by 2-N-Nonyl-hydroxy quinoline-N-oxide, amytal, or azide.

3 *Biochemistry*

MICROBIOLOGIC ASSAY FOR THYMINE USING A MUTANT OF STREPTOCOCCUS FAECALIS. V. M. Doctor. Univ. of Texas, Houston, Texas.

Streptococcus faecalis ATCC 8043 is not a suitable assay organism for thymine since much smaller concentrations of folic acid or citrovorum factor (CF) can replace the thymine requirement of this organism. A pad-plate assay method for thymine using this organism has been reported (Holmes and Welch. Proc. Soc. Exp. Biol. Med. 86, 564, 1954). In this method, the response of the organism to folic acid but not due to CF was prevented by addition of aminopterin to the basal medium. Streptococcus faecalis Akt, (Hutchison and Coultas. Proc. Am. Assoc. Can. Res. 2, 216, 1957) a mutant of Streptococcus faecalis ATCC 8043, requires both, folic acid and thymine for growth and can grow in absence of all other purines and pyrimidines. Studies have been conducted on a microbiologic assay method for thymine using this mutant. The basal medium is supplemented with 10 mγ/ml. folic acid. Under these conditions, the organism responds to 1 γ/ml. of thymine or a molar equivalent of thymidine. It does not respond to 100 γ/ml. of 5-hydroxymethyluracil, 5-hydroxymethylcytosine, dihydrothymine, cytosine, uracil, deoxyuridine, deoxycytidine, deoxycytidylic acid, adenine, guanine, xanthine, folic acid, CF and deoxyribonucleic acid. Fifty γ of 5-methylcytosine or 100 γ of thymidylic acid gives a growth response which is similar to that obtained by 1 γ of thymine.

4 *Pharmacology*

TERATOGENIC,URICOGENIC AND PROTECTIVE EFFECTS OF NICOTINAMIDE ANTAGONISTS AND SUBSTITUTES IN THE CHICK EMBRYO.
I.H. Krakoff*, C.R. Lacon* and D. A. Karnofsky. Sloan-Kettering Inst. for Cancer Research, New York, N.Y.

2-Ethylamino-1,3,4-thiadiazole (TDA) which increases uric acid synthesis in man, produces a similar effect in the developing chick embryo. This can be prevented by nicotinamide or dl-tryptophan and by the nicotinamide antagonists, 6-aminonicotinamide (6AN) and 3-acetylpyridine (3AP), 6AN and 3AP cause abnormalities in the embryo which can be prevented by nicotinamide, suggesting that they function as "antegonists" or "substitutes" for nicotinamide in different circumstances. Similar abnormalities are caused by unrelated substances including insulin which fails to inhibit the TDA effect and 3,3-dimethyl-1-phenyl triazene (DMPT) which appears to enhance it. The toxicity of DMPT is increased 20-fold by TDA. Diphosphopyridine nucleotide (DPN) measurements indicate that the TDA effect is not related to an influence on DPN synthesis. The variety of effects produced by these substances in different biologic systems and the dual role of 6AN and 3AP suggest that nicotinamide may function differently at different metabolic sites or in different organ systems.

5 *Nutrition*

VITAMIN SPARING BY ANTIBIOTICS. R.M. Barnes, C. Bourdeau*, E. Kwong* and G. Fiala*. Grad. School of Nutrition, Cornell Univ., Ithaca, N.Y.

Penicillin, bacitracin and chlortetracycline were compared for their sparing effect on vitamin B_{12}, pantothenic acid and thiamine in rats. A technique for the complete prevention of coprophagy was used to evaluate the importance of recycling feces as means of making newly synthesized vitamins available to the rat. Deficiency of the three vitamins as indicated by depressed growth rate was decreased by the antibiotics. There was some evidence that they were not equally effective. Chlortetracycline had less sparing action in pantothenic acid and thiamine deficiency than the other antibiotics. The sparing effect of the three antibiotics was completely blocked in the cases of vitamin B_{12} and thiamine, but only partially blocked for pantothenic acid when coprophagy was prevented. More extensive investigation of the sparing of thiamine by penicillin has shown that the antibiotic causes an increased fecal excretion of thiamine. Approximately 35% of the total fecal thiamine was utilized by the rat when coprophagy was permitted Penicillin did not alter the percentage utilization of fecal thiamine and, therefore, the entire sparing effect could be accounted for by the increased excretion of thiamine together with the practice of coprophagy.

6 *Nutrition*

THE INFLUENCE OF PARENTAL ASCORBIC ACID ON Ca^{45} UTILIZATION BY PROGENY IN THE DOMESTIC CHICKEN. P. A. Thornton (intr. by P. E. Waibel.) Colorado State Univ., Fort Collins, Colorado.

Parental ascorbic acid (20 mg per pound of ration) has been shown to markedly change the amount of Ca^{45} deposited in the bone to one hour post injection of rachitic progeny (Thornton et al., J. Nutrition, 69, P. 33). This study is a continuation of that work with observations being made from 30 minutes to 24 hours after injection of Ca^{45} into the lumen of the crop. The results indicated little difference in control progeny on the rate or amount of Ca^{45} deposited in the tibia from control or ascorbic acid supplemented parents. The rachitic progeny from the vitamin C fed parents showed a Ca^{45} deposition value and turnover rate somewhat similar to the two control groups. The rachitic progeny from control parents exhibited a much lower deposition value. The turnover rate of Ca^{45} in the bone for this group was also widely different from the three others showing a straight line response from 4 to 24 hours post injection. Epiphyseal plate measurements of rachitic chicks indicated that the progeny from ascorbic acid fed parents were more rachitic than those from control parents. These results indicated that calcium may be absorbed at a normal rate in the absence of vitamin D_3 when vitamin C is present in the parents' diet; however, the development of rachitic symptoms is evident.

7 *Immunology*

BACTERIOPHAGE NEUTRALIZATION BY SERUM. A. J. Toussaint (intr. by L. S. Baron) Dept. of Serology, VA Central Lab. of Clin. Path. and Res., Walter Reed Army Inst. of Res., Wash., D.C.

Neutralization of coliphages of the T series by normal human serum has been shown to depend upon the action of normal antibody of marked specificity and C' (Toussaint and Muschel, Nature 183:1825, 1959) in contrast to phage neutralization by antiserum which functions independently of C'. The similarity in specificity and the difference in C' requirement suggested further study of the two systems. Results indicated that immune and normal phage neutralizing antibodies have certain properties in common. The phage neutralizing activity (PNA) of immune and normal antibody is specifically blocked by soluble or particulate phage material. Both antibodies neutralize from 5×10^2 to 5×10^5 phage particles in accordance with the "percentage law". Bacteriophage neutralized by either antibody can be reactivated by papain. On the other hand, there are differences in the PNA of normal and immune sera which may reflect the difference in C' requirement. Immune antibody neutralized phage at 4°C; normal antibody has no activity at this temperature. The PNA of normal serum is inhibited by endotoxin which has no effect on the PNA of immune antibody. Thus, the data have indicated an essential similarity of both antibodies with the exception of the C' requirement by normal serum.

255. VITAMINS–Vitamin A

1 *Biochemistry*

THE ANTAGONISTIC ACTION OF VITAMIN A ON VITAMIN D TOXICITY. Irvin Clark and C. Andrew L. Bassett* Orth. Res. Labs & Dept. Biochem., Columbia, College of Physicians & Surg., N.Y., N.Y.

Toxic amounts of vitamins A & D were given to rats to increase the rate of bone resorption. Contrary to expectations, Vit. A ameliorated hypervitaminosis D. Vitamins were administered daily on a body weight basis. 60,000 u of Vit. D killed all rats within 3 weeks. Survival time was increased almost 2 weeks by adding 15,000 u of Vit. A. If 30,000 u of Vit. A were added, the rats lived at least 6 weeks. Despite the prolongation of life, weight loss began during the 1st week. Rats receiving 18,000 u of Vit. D gained weight for 3 weeks at which time some began losing weight. Administration of graded amounts of Vit. A together with the 18,000 u of Vit. D increased weight gain, the greatest gain occurring with the largest amount of Vit. A (30,000 u). Rats treated only with Vit. D showed extensive kidney calcification. Simultaneous administration of Vit. A markedly decreased calcification, and in some cases prevented it entirely. Calvaria and long bones of hypervitaminotic D animals showed evidence of advanced bone resorption. This resorption was prevented almost completely in rats receiving supplemental Vit. A. Implications of these findings will be discussed. (Supported in part by the Easter Seal Research Foundation and the American Cancer Society)

2 *Biochemistry*

THE EFFECT OF SODIUM GLYCOCHOLATE ON THE IN VITRO CONVERSION OF β-CAROTENE TO VITAMIN A ESTER. James Allen Olson. Univ. of Florida College of Medicine, Gainesville, Florida.

Upon intraduodenal injection of C^{14} labeled β-carotene into anaesthetized rats, intestinal vitamin A ester formation is maximal in less than an hour (Olson, J.A., Federation Proc. 18, 296 (1959). Recent studies have shown that bile, or more specifically, sodium glycocholate (0.4%), is required for the utilization of β-carotene by the intestine, in vivo and in vitro (Olson, J.A., Biochim. et Biophys. Acta, in press). In vitro suspensions of longitudinally cut intestinal segments were used for all subsequent experiments. Sodium deoxycholate, oleate, and cephalin were ineffective in promoting β-carotene utilization. On the other hand, the uptake and conversion of the presumptive intermediate, retinene, to vitamin A alcohol, and of vitamin A alcohol to ester proceed readily in the absence of glycocholate. Higher glycocholate concentrations (1%), however, inhibit β-carotene, retinene, and vitamin A alcohol utilization. The data suggest that glycocholate acts in a specific manner in the intestinal absorption of β-carotene rather than as an activator of an intracellular process leading to vitamin A ester formation. (The C^{14} vitamin A was kindly given by F. Hoffman-LaRoche and Co., Basle. This study was supported by PHS research grant No. A-1278 from the National Inst. of Arthritis and Metabolic Diseases.)

3 *Biochemistry*

ON THE ROLE OF VITAMIN A IN ADRENOSTEROID BIOSYNTHESIS. R. A. Van Dyke,* B. Connor Johnson, and G. Wolf. Dept. of Animal Science, University of Illinois, Urbana.

Previous work has shown an interruption in the production of total adrenal steroids in vitamin A deficiency (Fed. Proc. 17, 327 (1958). In order to determine which steroids are affected radio-cholesterol and radio-mevalonic acid were used as precursors of adrenal steroids in in vitro experiments with rat and pig adrenal homogenates. ATP, TPN, DPN, glucose and 1 μc of cholesterol-4-C^{14} (16γ) or of mevalonic acid-2-C^{14} (4.1 mg.) were added to each incubation mixture. The steroids were extracted and separated by paper chromatography. A lowered production of corticosterone from mevalonic acid was found in adrenal homogenates from vitamin A-deficient rats. Using cholesterol as substrate, there was also a lowered synthesis of corticosterone, as well as of desoxycorticosterone and progesterone, in both rat and pig vitamin A-deficient adrenal homogenates. In addition, a markedly increased labeling of the 17-hydroxy steroids was found in the vitamin A-deficient pig adrenal experiments. From this work it can be concluded that there is a lower conversion of cholesterol to progesterone and corticosterone in the adrenals of vitamin A-deficient animals but an increased conversion of cholesterol to the 17-hydroxy steroids, indicating a path to the latter which does not go through progesterone.

4 *Nutrition*

UTILIZATION OF CAROTENE AS EFFECTED BY VARIATIONS IN DIETARY NITROGEN IN YOUNG RATS. V. Johnson* and L. Arnrich. Food and Nutrition, Iowa State University, Ames, Iowa.

Both quantity and quality of protein have been shown to affect utilization of carotene by the growing rat. Hepatic and renal vitamin A were determined in rats fed carotene as the sole source of vitamin A and given the following variations in dietary protein: (1) casein or lactalbumin at levels of 5, 20, 40, and 60%, (2) methionine added in amounts of .5, 1? and .7% to 5, 20 and 40% casein diets respectively, and (3) 15% glycine added to a 5% casein diet. Food intake was equalized in all experiments. A linear increase in hepatic vitamin A was found with an increase of protein in the diet up to but not beyond 40%. Casein and lactalbumin gave similar vitamin A stores at comparable levels of intake. This linear increase could not be attributed to the increased amounts of methionine consumed at the higher protein levels. Nor did the increase in amino nitrogen in (3) improve carotene utilization. However fecal losses of carotene were inversely related to protein intake, so that part of the effect of protein on carotene utilization might be accounted for by its effect on absorption of carotene.

256. VITAMINS–Vitamin B

1 *Biochemistry*

THE BIOLOGICAL ACTIVITY OF 6-ETHYL-9-(1'-D-RIBITYL)-ISOALLOXAZINE. J.P. Lambooy. Dept. of Physiol., Univ. of Rochester, Rochester, N.Y.

In contrast to 6,7-diethyl-, 6-methyl-7-ethyl-, and 6-ethyl-7-methyl-9-(1'-D-ribityl)-isoalloxazine, all of which were essentially equal to riboflavin for the nutrition of L. casei, 6-ethyl-9-(1'-D-ribityl)-isoalloxazine has approximately 3% of the activity of riboflavin. 6,7-Diethyl-flavin is an inhibitor (I.I.=6). Both the 6-methyl-7-ethyl-flavin and 6-ethyl-7-methyl-flavin are vitamin-like in that they stimulate "real"growth in the riboflavin-deficient rat. 6-Ethyl-flavin is almost completely devoid of activity. When given to a deficient animal at levels of 5 to 80 γ per day no growth resulted. When the 6-ethyl-flavin was given at 2 mg. per day the animals failed to grow and 8 of 10 died. On 2 mg. plus 1 γ of riboflavin, the animals also failed to grow but only 4 of 10 died. On 2 mg. plus 5 γ of riboflavin all animals survived and the growth was equal to that of animals receiving 1 γ of riboflavin and about 1/2 of that shown by animals receiving 5 γ of riboflavin. The inhibition index is approximately 400. (Supported by a grant from U.S.P.H.S. #CY-2940.)

2 *Biochemistry*

ENZYMATIC AND NON-ENZYMATIC DECARBOXYLATION OF α-KETO ACIDS. Elliot Juni. Dept. of Bacteriology. Emory Univ., Atlanta, Ga.

It has been proposed that two enzymatic sites are involved in the decarboxylation of pyruvate by α- carboxylase (Juni, E., Bacteriol. Proc., 113, 1955). At the first site pyruvate is decarboxylated to an aldehyde-thiamine-PP complex. The aldehyde moiety is then irreversibly transferred to the second site where reversible dissociation to free aldehyde occurs. α-Carboxylases produce free acetaldehyde in contrast to the non-enzymatic pyruvate decarboxylation catalyzed by thiamine where α-acetolactate is formed exclusively (Koffler, H. and Krampitz, L. O., Bacteriol. Proc., 113, 1955). Acetaldehyde and glyoxylate have been shown to be second site inhibitors of yeast carboxylase. Using concentrations of these aldehydes which completely inhibit acetaldehyde formation from pyruvate, enzymatic decarboxylation of higher α-keto acids to free aldehydes still occurs. This finding is consistent with the concept that aldehyde-thiamine-PP complexes of aldehydes having chain lengths greater than acetaldehyde are relatively unstable and decompose spontaneously to free aldehydes, thus avoiding the requirement for the second enzymatic site. Evidence in support of this hypothesis is the demonstration that higher α-keto acids are decarboxylated, in part, to free aldehydes in the non-enzymatic model system containing thiamine.

3 *Biochemistry*

PURIFICATION AND PROPERTIES OF PYRIDOXAL KINASE FROM RAT LIVER. Donald B. McCormick* and Esmond E. Snell, Dept. of Biochemistry, Univ. of California, Berkeley.

Kinases which convert vitamin B_6 to its phosphates are of almost ubiquitous occurrence. Until recently, only the kinase from yeast had been studied in reasonable detail (Hurwitz, J. Biol. Chem., 205, 935 (1953)). An improved assay for pyridoxal phosphate based on that of Wada, et al. (J. Vitaminol. (Japan) 3, 183 (1957) has now been developed. With its aid, the kinase from rat liver has been purified 750-fold by treatment with heat, $(NH_4)_2SO_4$, acid, alumina gel, and DEAE-cellulose. Kinetic studies indicated a protracted zero-order reaction rate which was enhanced in crude preparations by endogenous adenylate kinase. Pyridoxal (K_s, 1.5×10^{-5}M) was the preferred substrate, but pyridoxine (K_s, 2.5×10^{-5}M) pyridoxamine (K_s, 1.5×10^{-4}M) and several analogs were also phosphorylated at the pH optimum of 5.75. ATP (K_s, 3.5×10^{-5}M) was required specifically as the phosphate donor. Zn^{++}, Mg^{++}, and Mn^{++} served as activating cations, but only Zn^{++} ($K = 1.0 \times 10^{-6}$M) activated maximally. Like the pyridoxal phosphokinase of brain (McCormick and Snell, Proc. Nat. Acad. Sci., 45, 1371 (1959)), the liver kinase was inhibited strongly by hydrazines and other carbonyl reagents and their condensation products with pyridoxal. (Supported in part by a grant from the National Institutes of Health, U.S. Public Health Service).

4 *Biochemistry*

ASPARTATE CARBAMYL TRANSFERASE AND BIOTIN. M. A. Grillo, and S. P. Mistry (intr. by T. S. Hamilton). Dept. of Animal Sci., Univ. of Illinois, Urbana.

We have studied the role of biotin in the synthesis of ureidosuccinic acid from aspartate and carbamyl phosphate, using enzyme preparations from the livers of normal and biotin-deficient rats. The average activity per mg. protein determined at two enzyme concentrations was not significantly altered in the case of the following groups of animals: (1) 20% spray-dried raw egg white diet, (2) 20% egg white diet with weekly 100 μg biotin injection, (3) 20% egg white diet with weekly injection of biotin plus injection 48, 24 and 4 hours before the experiment, and (4) commercial stock ration plus biotin injection 48, 24 and 4 hours before the experiment. Preincubation of the enzyme with avidin also did not significantly alter the specific activity of the preparation. These results differ from those obtained with cell-free bacterial systems where a role of biotin in carbamylation reactions has been demonstrated.

5 *Biochemistry*

NICOTINAMIDE AND NICOTINIC ACID UPTAKE BY ASCITES TUMOR CELLS. L. S. Dietrich. Univ. of Miami School of Med., Coral Gables, Fla.

The uptake of low levels (3.7×10^{-5} M final concentration) of nicotinamide-7-C^{14} and nicotinic acid-7-C^{14} was studied employing Ehrlich, Sarcoma 180 and 755 Adenocarcinoma ascites tumors. At 4° the nicotinamide concentration of the intracellular fluid of these cells approached the concentration of the extracellular water. At this temperature nicotinic acid permeated these ascites cells to a far lesser extent. At 30° the intracellular concentration of nicotinamide was approximately twice that of the extracellular fluid. At this temperature the intracellular concentration of nicotinic acid was equal to and 3 times that of the extracellular water of the Ehrlich and 755 tumor, respectively. In all cases equilibrium was reached very rapidly, the intracellular concentration of the vitamin being the same at 1 and 6 minutes after addition. Under these conditions the labeled nicotinamide or nicotinic acid within the cell is almost completely in the free state. These data are interpreted as meaning that both nicotinamide and nicotinic acid are actively taken up by ascites cells and that the efficiency of this reaction varies with the cell type.

6 *Biochemistry*

ROLE OF BIOTIN IN THE SYNTHESIS OF ORNITHINE TRANSCARBAMYLASE Joanne Ravel and William Shive, Clayton Foundation Biochemical Institute, Dept. of Chemistry, Univ. of Texas, Austin.

Although biotin deficiency in S. lactis 8039 is associated with a decrease in the ornithine transcarbamylase content of the cells, highly purified enzyme preparations do not contain significant amounts of biotin, indicating an indirect role of biotin. In an investigation of the components of the enzyme which might be related to biotin, a tryptic digest of the purified enzyme was found to replace the biotin requirement for synthesis of the enzyme. However, tryptic digests of casein have similar activity suggesting a peptide effect. Of a group of synthetic peptides, glycylasparagine largely replaces the tryptic digests. Other peptides, e.g. glycylmethionine and alanylphenylalanine, augment the effect of glycylasparagine so that a mixture of peptides replaces the biotin requirement. Increased aspartate does not replace glycylasparagine, but omission of aspartate even in the presence of biotin diminishes enzyme synthesis. The role of biotin in this system apparently is in the production of an active 4 carbon unit. Inability to assimilate adequate amounts of exogenous aspartate may account for the diminished synthesis not only of this enzyme but also of certain other enzymes which are affected by a biotin deficiency but which do not contain biotin.

XUM

7 *Pharmacology*

VITAMIN B_6 AND ISONIAZID TOXICITY. R.W. Manthei. The Jefferson Medical College of Philadelphia, Pennsylvania.

Isoniazid (INH) has been shown to interfere with the biological action of vitamin B_6. Some aspects of INH toxicity can be reversed by pyridoxine administration. In studies with adult mice, increasing protection against a lethal injection of 200 mg/kg of INH was obtained with pyridoxine in doses from 25 to 150 mg/kg. Pyridoxal, however, in doses above 25 mg/kg increased the toxicity of sublethal doses of INH. Both pyridoxal and pyridoxine offered slight protection (10-30%) against 200 mg/kg of INH when given in doses from 0.5 to 25 mg/kg. In studies with weanling mice (10Gm), 0.5 to 1 mg/kg of pyridoxal produced 80 to 100% protection against 200 mg/kg of INH. Pyridoxine at 1 mg/kg gave only 20 to 40% protection against this dose of INH. Still less protection was achieved with pyridoxine at doses lower than 1 mg/kg or at higher doses up to 25 mg/kg. Above 25 mg/kg pyridoxine offered complete protection as it did in adult mice. The pyridoxal hydrazone of INH was not toxic to mice in doses equivalent to 200 mg/kg of pyridoxal and INH. The data indicate that pyridoxal can offer greater protection than pyridoxine when given in doses in the vitamin therapeutic range while pyridoxine, possibly by a different action, protects against the acute toxicity of INH when given in large doses. (Supported by N.I.H. grant B-1084)

8 *Nutrition*

EFFECTS OF PYRIDOXINE DEPLETION ON THREE ENZYMES REQUIRING PYRIDOXAL PHOSPHATE (B_6.PO_4) AS A COFACTOR. **Fred Rosen and Richard Milholland*. (Dept. of Experimental Therapeutics, Roswell Park Memorial Inst., Buffalo, N. Y.)**

The activities of glutamic acid decarboxylase (GDC), and glutamic-pyruvic (GPT) and glutamic-oxaloacetic (GOT) transaminases were measured at biweekly intervals in the brain and liver of rats fed a B_6-deficient diet. Endogenous GDC activity in brain is about 40 per cent of that measured in the presence of B_6.PO_4, whereas the endogenous values for GPT and and GOT in brain and liver are not changed by the addition of cofactor. Pyridoxine depletion for 2 weeks decreased the activity of GDC in brain to 60 per cent and after 8 weeks to 40 per cent of the endogenous level; depletion for the same periods lowered GPT activity in liver to 70 and 50 per cent of normal, respectively, while GOT activity decreased only to 80 per cent of normal. Injection of B_6-deficient animals with pyridoxine (2 mg/rat) or its congeners restored GDC, GPT and GOT activities to their normal endogenous values. Hepatic GPT activity, but not GOT, increases progressively with age. For each enzyme studied, the apoenzyme level was not altered by the deficiency. Thus, differential effects rather than uniform inhibition of these enzymes occur during depletion of pyridoxine or following treatment with certain B_6 antagonists (Fed. Proc. 18: 543, 1959).

9 *Nutrition*

THE EXCRETION OF N-METHYL-4-PYRIDONE-5-CARBOXAMIDE IN BOTH HUMAN AND MONKEY URINE. M. L. Wu Chang. Division of Animal Nutrition, University of Illinois, Urbana.

N-methyl-4-pyridone-5-carboxamide was found to be the major normal metabolite of nicotinic acid in rat urine (Chang and Johnson, J. Biol. Chem. 234, 1817, 1959) and to be derived from pyridine nucleotide (Chang, Fed. Proc. 18, 520, 1959). To determine whether this compound is also excreted by man, four men and four women were used as subjects and 24-hour urine samples were collected. Approximately 1.3×10^4 cpm each of C^{14}-N-methyl-4-pyridone-5-carboxamide (4-P) and C^{14}-N-methyl-2-pyridone-5-carboxamide (2-P) were added to each urine sample before analysis. Analysis was carried out by isolation of each compound and determination of isotope dilution. The results indicated that female subjects excreted from 5 to 7 mg. of 4-P daily and male subjects excreted 10 to 25 mg. The amount of 2-P was higher than that of 4-P in both sexes. C^{14}-nicotinic acid was injected intramuscularly into a monkey and labeled 4-P was found in the urine, in addition to labeled N-methylnicotinamide, nicotinuric acid, nicotinic acid, 2-P, and nicotinamide.

10 *Nutrition*

RETENTION OF THIAMINE IN HEAT PROCESSED MEATS. C. H. Lushbough and Barbara S. Heller (intr. by B. S. Schweigert.) American Meat Inst. Fdn. and Dept. of Biochemistry, University of Chicago, Chicago, Illinois.

This study was undertaken to evaluate the effects of various methods of heat processing upon thiamine retention in meat. Beef round, uncured ham, lamb leg, and chopped pork luncheon meat were oven roasted, electronic oven roasted, steam retorted, or autoclaved, under varying conditions, to desired internal temperatures. Sample extracts for thiamine determination were prepared (METHODS OF VITAMIN ASSAY, 1947, p. 77), and microbiological thiamine assays performed (Deibel et al., J. Bact., 74: 818, 1957). Thiamine retentions ranged from 75% to 103% for meats roasted at 200° or 300°F. oven temperature; 60% to 86% at 400°F., or with the use of an electronic oven; 26% to 67% for steam retorted meats; and 0% to 5.4% for meats autoclaved 4 or 16 hours. The results obtained with varying time and temperature relationships during cooking confirm reports that thiamine destruction is progressive and continuous throughout the heat processing period. Thus the use of lower temperatures and shorter processing schedules results in increased thiamine retention in meats. (Supported in part by a grant in aid from the National Live Stock and Meat Board.)

11 *Nutrition*

B-VITAMIN DEFICIENCY IN THE MATURE TURKEY HEN. T. M. Ferguson, R.L. Atkinson*, R.H. Mitchell*, M.L. Jones* and J. R. Couch. Texas A&M College System, College Station, Texas.

A casein-gelatin diet, complete in known essentials or deficient in folic acid, riboflavin, pyridoxine, biotin, pantothenic acid, niacin or vitamin B_{12} was fed to mature Beltsville Small White turkey hens throughout a 24 week period. Anemia was found in the folic acid and pantothenic acid groups at six weeks. At twenty weeks, all groups deficient in one of the B vitamins had lowered red cell counts. The control group maintained a normal erythrocyte level. Half of the hens fed the riboflavin deficient diet were dead by 14 weeks. Dermatitis, at the angles of the mouth, and scaliness of the legs and skin were observed in the riboflavin and pantothenic acid deficient groups. Hatchability of eggs was zero in the biotin and riboflavin deficient groups after four weeks and in the pantothenic acid group after six weeks.

12 *Nutrition*

INTRACELLULAR DISTRIBUTION OF CARBOXYL-C^{14}-BIOTIN IN RAT AND CHICK LIVERS. S. P. Mistry and K. Dakshinamurti (intr. by T. S. Hamilton). Dept. of Animal Science, Univ. of Illinois, Urbana.

One microcurie (10 μg) of radiobiotin per 100 gm. body wt. was injected into biotin-deficient rats and chicks which were killed 1, 2 and 3 hours later. In each case recovery was about 80% and a maximum of 14% of the dose was found in liver. Approximately one-third of the dose was excreted, whereas expired CO_2 and viscera each contained about 5%. Very little activity was found in heart, lung and spleen. When the livers were fractionated by differential centrifugation, 39-53% of the activity of the homogenate was found in the supernatant, 18-29% in the mitochondria, 15-23% in nuclear material, and only about 2% in the microsomes. The percentage of protein in the fractions generally followed the distribution of radioactivity. About 90% of the biotin in the cellular fractions of normal rat liver is in the bound form, except in the microsomes, where nearly 80% is present as free biotin and biocytin. Deficient liver contains five to ten times less total biotin than does normal liver. A relatively large amount of biotin in the deficient liver is retained in the nuclear material. This is in general agreement with our preliminary findings (Fed. Proc. 17: 277, 1958). The results of the fractionation of normal supernatant will be reported.

13 *Nutrition*

EFFECT OF DIETARY LEVEL OF NIACIN AND TRYPTOPHAN ON BLOOD AND LIVER PYRIDINE NUCLEOTIDE CONCENTRATIONS. Mary A. Morrison* and A. E. Harper. Dept. of Biochemistry, Univ. of Wisconsin, Madison.

Male weanling rats weighing 40-50 grams were fed a niacin-free basal diet containing 6% of casein and 12% of gelatin for a two week experimental period. When graded levels of tryptophan were added to the diet, a progressive increase in growth occurred with levels of tryptophan up to 0.06% of the diet. Both blood and liver pyridine nucleotide concentrations increased in response to increments of tryptophan, but the increases were more pronounced in blood than in liver. The addition of niacin (2.5 mg%) exerted a sparing effect on tryptophan for growth when the dietary level of tryptophan was suboptimal but not when it was adequate. Increments of niacin (0, 0.5, 10, 250 mg%) caused blood pyridine nucleotide concentrations to rise regardless of the dietary level of tryptophan but the increases with the lower levels of niacin were slight when the tryptophan intake was adequate. The highest level (250 mg%) of niacin gave a large increase in liver pyridine nucleotide concentration with each dietary level of tryptophan but the other levels were most effective when the tryptophan level was suboptimal for growth.

14 *Nutrition*

Effect of Pyridoxine, Pantothenic Acid, and Thiamine Deficiencies upon Antibody Formation to PR8 Influenza Virus in Rats. Sarah Hopper* and A.E. Axelrod. Biochemistry Dept., School of Medicine, Univ. of Pittsburgh, Pittsburgh, Pa.

Previous studies in our laboratory have demonstrated the deleterious effects of a variety of vitamin deficiency states in the rat upon antibody response to the antigenic stimuli of human erythrocytes and diphtheria toxoid. The present investigation deals with comparable studies in which PR8 influenza virus was utilized as antigen. Male, weanling rats of the Holtzman strain were rendered deficient in pyridoxine, pantothenic acid or thiamine and subsequently immunized with intraperitoneal injections of allantoic fluid infected with PR8 influenza virus. Resulting levels of serum antibody were determined by a standard hemagglutination-inhibition procedure. Both ad libitum-fed and inanition control rats were immunized in a similar fashion. High levels of serum antibody were attained in both ad libitum-fed and inanition control rats. In contrast, antibody production was markedly impaired in pyridoxine and pantothenic acid-deficient animals. Although morbidity of thiamine-deficient rats was very marked, impairment of antibody production was not observed. It is concluded that antibody production to the antigenic stimulus of PR8 influenza virus in rats requires an adequate intake of pyridoxine and pantothenic acid. (Supported by a grant from the U.S.P.H.S.)

15 *Nutrition*

SOME DRUG-VITAMIN INTERRELATIONSHIPS IN MICROORGANISMS, J.M. McLaughlan*, K.G. Shenoy*, and J.A. Campbell, Food and Drug Lab. Dept. of Natl. Health and Welfare, Ottawa, Canada.

Clinical evidence suggests that certain drugs used over extended periods in man produce specific vitamin deficiencies. In this work Lactobacillus leichmannii and Tetrahymena pyriformis have been used to screen 50 drugs for possible anti-vitamin effects. The effect of a drug on response to individual vitamins served as the criterion of antivitamin activity. Phenformin appeared to have antifolic acid activity for L. leichmannii but not for the protozoan. Chlorpromazine, promazine, chloroquin, methapyrilene hydrochloride and methantheline bromide, appeared to have antipyridoxal activity for T. pyriformis. Propylthiouracil, tolbutamide, methahexamide and chlorpropamide were found to have antipantothenate activity for the protozoan but not for L. leichmannii. The rate of growth of T. pyriformis was markedly reduced with the sulfonylurea drugs but the effect was reversed by increasing the concentration of pantothenate. Neither phenformin nor the common sulfa drugs exhibited antipantothenate activity. It is known that the sensitivity to insulin is increased in pantothenate deficient rats (Endocrinol, 60: 388, 1952); the present data suggest a possible mechanism of the hypoglycemic action of propylthiouracil and the sulfonylurea drugs.

16 *Nutrition*

INTAKE AND URINARY EXCRETION OF FOLIC ACID BY 36 PREADOLESCENT GIRLS. June K. Pace, Larue B. Stier, Doris D. Taylor, and Patricia A. Goodman (intr. by Elsie Z. Moyer). Human Nutrition Research Division, USDA, Washington, D. C.

Average daily intake and urinary excretion of folic acid was determined by microbiological assay for 36 healthy girls 7 to 9 years of age in cooperative work with the Southern Region. Three metabolic studies were done: one in fall 1954 with 11 girls for 64 days in Tennessee and Louisiana, one in summer 1956 with 12 girls for 56 days in Virginia, and one in summer 1958 with 13 girls for 48 days in Virginia. Diets in 1954 and 1956 were made up of usual foods with nutrients at levels recommended by the NRC for children of this age. Diets in 1958 contained less protein and were supplemented with minerals and vitamins. The average daily intake of folic acid was 97, 80, and 52 micrograms in 1954, 1956, and 1958, respectively. Average daily urinary excretion of folic acid was 1.24, 1.13, and 1.46 micrograms, respectively. The average amount of folic acid excreted was less than 5 percent of the intake. Urinary excretion of folic acid did not appear to be affected by the amount of folic acid or protein in the diet, or size of the child.

17 *Nutrition*

EFFECT OF FEEDING PATTERN AND VITAMIN B_6 DEFICIENCY ON ACETATE INCORPORATION INTO LIPIDS IN RAT LIVER SLICES. Mary Ann Williams and Ruth Pertel*. Department of Nutrition and Home Economics, University of California, Berkeley.

Vitamin B_6 deficiency has been found to reduce liver CoA concentration in rats by 50% (Williams and Hata, Arch. Biochem., 80, 367, 1959). To try to determine whether lipid metabolism was altered by the decrease in liver CoA, lipid synthesis from acetate-2-C^{14} was studied in liver slices from vitamin B_6-deficient and control rats. Vitamin B_6-deficient rats were allowed food for 8 hours during the day or for 24 hours. Pair-fed controls were fed once and twice daily.

The development of vitamin B_6 deficiency was not significantly affected by the difference in feeding schedules. Total incorporation of C^{14} into total lipids was similar in pair-fed controls given food once and twice daily. Vitamin B_6-deficient rats with 8-hour access to food incorporated less label into total lipid than did deficient rats with 24-hour access to food, presumably since the former had been without food for 18 hours before sacrifice. However, slices from vitamin B_6-deficient rats with 8-hour access to food still incorporated more C^{14} into total lipid, especially into digitonin-precipitable material, than did slices from pair-fed controls fed twice daily to simulate the feeding rate of the deficient rats. (Supported by Grant A-1189C, PHS, NIH).

18 *Nutrition*

TISSUE RETENTION AND URINARY EXCRETION OF INJECTED VITAMIN B_{12}* AND ITS COENZYME IN THE RAT. Vernon Wong, Samuel Yeh and Bryant Chow (intr. by E.B. McCollum.) Johns Hopkins Univ., School of Hygiene & Public Health, Balto., Md.

Coenzyme-B_{12} (Co-B_{12}) possesses certain biological activity of B_{12}. Two groups of adult rats were admin. intraperitoneally different dosages of these 2 vits. and were sacrificed 36 hrs. afterwards. The radioactivity in the organs and 24 hr. urine was measured. Data show: 1) radioactivity in urine and liver and kidneys increased with increasing dosage except in brain which was approx. the same; 2) radioactivity in eyes was negligible; 3) the ratio of radioactivity in kidney to liver is higher in B_{12} than in Co-B_{12} group.

	Dosage B_{12} (mmcg)	Kidneys	Liver	Kid./Liv.	Urine
B_{12}	80	10	4.1	2.6	2.2
	240	32	8.5	3.7	5.3
	800	110	20.9	5.4	20.9
Co-B_{12}	80	2.9	8.9	0.3	2.9
	200	12.4	10.3	1.2	3.8
	600	26.9	19.1	1.4	13.4
	2000	81.6	53.6	1.5	138.7

XUM

19 *Nutrition*

DEFICIENCIES OF CERTAIN B-VITAMINS ON ERYTHROCYTE AND TISSUE GLUTATHIONE IN RATS. Jeng M.Hsu, Robert L. Duvall* and Bacon F. Chow. V.A.Hosp. and Johns Hopkins Univ.,Baltimore.

Glutathione(GSH) is a coenzyme involved in oxidative and reductive system and its content in erythrocytes of rats and chicks is decreased in B_{12} deficiency. The effect of deficiencies of other B-vitamins in rats is now reported. Weanling and young adult rats of both sexes were fed a casein surcose diet with or without the supplementation of the test vit. The conc. of GSH in blood and tissues were estimated by nitroprusside test. Our data indicate that B_6 deficiency elevates GSH content in both erythrocyte and liver(343±17.0, 298±13.8 uM/100 ml RBC; 663±45.9,388±24.6 uM/100 g of wet tissue for def. and treated groups, resp.). Admin. of desoxypyridoxine to B_6 def. rats further exaggerated the dif. of liver GSH activity. However, thiamine def. decreases the conc. of GSH in erythrocytes and increases the liver GSH level(176±13.1, 321±21.9 uM/100 ml RBC; 715±31.9, 389±59.5 uM/100 g of wet tissue for def. and treated groups, resp.). Moreover thiamine def. results in a 46% decrease of GSH activity in cardiac muscle, but has no effect on the level of GSH in kidneys. Deficiencies of riboflavin and pantothenate in rats, however, does not effect GSH level in blood and tissue.

20 *Nutrition*

EFFECTS OF NICOTINAMIDE AND RELATED COMPOUNDS ON THE ANTI-LEUKEMIC ACTIVITY OF 2-AMINO-1,3,4-THIADIAZOLE. Herbert F. Oettgen*, J.A. Keppert*, V. Coley*, and J. H. Burchenal. Sloan-Kettering Institute, New York, New York.

The activity of 2-amino-1,3,4-thiadiazole (TDA) against transplanted mouse leukemia B82T at 40 mg/kg/day (95-100% tumor inhibition) has been reversed by nicotinamide at 2.4 to 24 mg/kg/day, and nicotinic acid at 23 mg/kg/day. Essentially complete reversal of the inhibitory effect was also produced by 3-acetyl-pyridine at 2.4 to 121 mg/kg/day, DPN at 13 to 133 mg/kg/day, and 6-aminonicotinamide at 2.8 mg/kg/day. Somewhat less reversal was shown by 5-fluoronicotinamide at 28 mg/kg/day, 5-aminonicotinamide at 28 mg/kg/day and 5-methylnicotinamide at 27 mg/kg/day. No significant reversal of TDA activity was seen with 2-fluoronicotinamide at 28 mg/kg/day, 6-fluoronicotinamide at 28 mg/kg/day, 5-fluoronicotinic acid at 27 mg/kg/day, D-tryptophane at 204 mg/kg/day and L-tryptophane at 41 mg/kg/day. The fact that nicotinamide antagonists like 3-acetyl-pyridine, 6-aminonicotinamide and 5-fluoronicotinamide at certain doses appear to reverse the anti-leukemic activity of another nicotinamide antagonist suggests a different site of action of these compounds.

21 *Nutrition*

NIACIN BIOSYNTHESIS IN THE DEVELOPING CHICK EMBRYO. Robert G. Wilson and L. M. Henderson. Dept. of Biochemistry, Okla. State Univ., Stillwater, Okla.

Previous non-isotopic studies from several laboratories have indicated that quinolinic acid is not involved in niacin synthesis in the chick embryo. In the experiments to be reported tryptophan-7a-C^{14}, 3-hydroxyanthranilic acid-H^3 and quinolinic acid-H^3 were injected into developing chick embryos to determine the extent to which these compounds serve as sources of nicotinic and quinolinic acids. The first experiment covered the 12th to 18th days of incubation and the second the 10th to 22nd days. The isotope from all three compounds was incorporated into the nicotinic acid and quinolinic acid isolated. In the two experiments the isotope in the nicotinic acid from the tryptophan-7a-C^{14}-injected embryos was diluted 262 and 160 times respectively. The corresponding dilutions in the quinolinic acid of the embryos were 98 and 69 in the two experiments. The dilutions of isotope from 3-hydroxyanthranilate-H^3 in forming nicotinic acid were 311 and 48.5 and for quinolinic acid 78 and 21. Dilutions of 72 and 12.3 were observed in the conversion of quinolinic acid-H^3 to nicotinic acid in the two experiments. The isotope in the quinolinic acid in the embryos was diluted 3.8 and 1.8 times compared to the quinolinic acid-H^3 that was injected.

257. VITAMINS–Vitamins B–Vitamin B_{12}

1 *Physiology*

BINDING OF Co^{60}-VITAMIN B_{12} BY LACTOBACILLUS LEICHMANII. S. Kashket and June L. Tave (intr. by A. Leaf.) Massachusetts General Hospital and Harvard Medical School, Boston, Mass.

The accumulation of Co^{60}-labeled vitamin B_{12} by Lactobacillus leichmanii (ATCC 7830), an organism that requires vitamin B_{12} for growth, was observed and studied. Growing and non-growing cells accumulate B_{12}. The reaction follows first-order kinetics with total uptake proportional to the number of cells present. Uptake is unaffected by temperature between 4° and 4[illegible] and by various metabolic inhibitors, but is inhibited by heating to 100° and by ethanol treatment. Release of radioactivity from cells after addition of unlabeled B_{12} indicates that binding is reversible. The affinity constant is approximately 10^{9}. Maximal binding occurs at a B_{12} concentration of 10 mμg. per ml although growth (as measured by turbidity) is maximal at 0.02 mμg. per ml. Lactobacillus delbrueckii exhibits similar binding properties; Lactobacillus casei does not. Bound B_{12} is taken into the ribosomes and soluble fraction of the cell, but not into the cell wall and membranes. In the soluble fraction, B_{12} exists in freely dialyzable form. Further studies on the B_{12} binding sites are in progress. (Supported by a grant from the American Cancer Society.)

2 *Biochemistry*

PLASMA DISAPPEARANCE AND TISSUE UPTAKE OF ANIMALS INJECTED WITH $Co^{60}B_{12}$. Harold L. Rosenthal. Washington University, St. Louis, Mo.

Within 48 hours following I.V. or I.M. administration of $Co^{60}B_{12}$, 90 per cent of the dose leaves the blood stream. During the succeeding 26 days, two disappearance curves were found for doe rabbits and hens and roosters. In male rabbits and male human subjects only one disappearance curve was found. The rapidly disappearing component A in both sexes of rabbits and chickens, (T/2=4.2 days)is similar to that found in human subjects (T/2=5.7 days). The more slowly disappearing component B in doe rabbits and chickens of both sexes approximated an apparent biological half life of 29.9 days. The apparent half life of component A compares favorably with calculated values of 6.0 days (Reizenstein, Acta Med. Scand. 165 1, 1959).In male and female dogs, the plasma disappearance rate was very rapid (T/2=1.5 days). Tissue radioactivity of chickens and rabbits 26 days post injection were considerably different. Chicken:rabbit tissue values for liver, 5.4; kidney, 5.0; heart, 2.8; spleen, 3.1; and leg muscle, 0.5 suggest that most chicken tissues are highly unsaturated with respect to cyanocobalamin. (Supported by a grant from U.S.P.H.S.)

3 *Biochemistry*

A VITAMIN B_{12} PROTEIN REQUIRED FOR METHIONINE SYNTHESIS IN EXTRACTS OF ESCHERICHIA COLI. R. L. Kisliuk (intr. by M. Silverman.) Natl. Insts. of Health, Bethesda, Md.

A vitamin B_{12} protein required for methionine synthesis from homocysteine and serine has been purified 35 fold from sonic extracts of a glycine or serine requiring mutant of E. coli grown on minimal medium supplemented with glycine (0.3 g/l.) and vitamin B_{12} (2 ug/l.). The purified product contains 0.4 ug B_{12} per mg protein as assayed with Lactobacillus leichmannii and has an absorption maximum at 405 mμ at pH 7.8. In contrast to the B_{12} coenzymes described by Barker, Weissbach and co-workers, which do not stimulate methionine synthesis in the system employed, the B_{12} protein is not light sensitive and is relatively insensitive to cyanide treatment. No B_{12} protein can be detected in extracts of cells grown on minimal medium plus glycine and such cells contain very little assayable B_{12} (20 mμg/gm acetone-dried cells). The results are consistent with the hypothesis (Kisliuk and Woods, Biochem. J. in press) that organisms grown in the presence of vitamin B_{12} possess a mechanism for methionine synthesis which differs from that in organisms grown in the absence of the vitamin.

4 *Biochemistry*

ISOLATION AND PROPERTIES OF NEW COBAMIDE COENZYME ANALOGUES. J. I. Toohey* and H. A. Barker, Dept. of Biochemistry, Univ. of California, Berkeley, Calif.

Cobamide coenzymes containing adenine, benzimidazole or 5,6-dimethylbenzimidazole in the nucleotide and possessing activity in the enzymatic system converting glutamate to β-methylaspartate have been previously isolated from cells of Clostridium tetanomorphum or Propionibacterium shermanii (J. Biol. Chem., 235, No.1 and 2, 1960). Several new cobamide coenzyme analogues have been isolated from cells of P.arabinosum exposed to 2,6-diaminopurine, 5-nitrobenzimidazole, 5-methylbenzimidazole, or 5-trifluoromethyl-1,2-diaminobenzene. The cells generally yield two different cobamide coenzymes that can be separated on a Dowex-50 column. Like other cobamide coenzymes, the new analogues are inactivated by exposure to light or cyanide ion. The coenzymes containing a purine in the nucleotide moiety are inactivated by cyanide more rapidly than those containing a benzimidazole. Treatment with cyanide releases one mole of adenine and a dicyanocobamide containing the characteristic heterocyclic base. Acid hydrolysis of the cobamides liberates the bases which have been isolated and characterized. The Km values and the molar activities of the new analogues in the enzymatic assay fall between those of the cobamide coenzyme containing benzimidazole (most active) and 5,6-dimethylbenzimidazole (least active).

5 *Biochemistry*

FORMATION IN VITRO OF AN ENZYME OF METHIONINE BIOSYNTHESIS FROM VITAMIN B_{12} AND APOENZYME. S. Takeyama* and J.M. Buchanan. Div. of Biochemistry, Mass. Institute of Technology, Cambridge 39.

One of the essential enzymes for synthesis of the methyl group of methionine from formaldehyde or the β-carbon of serine has been purified from E. coli strain 113-3 (which requires B_{12} or methionine for growth) cultured on vitamin B_{12} (Fed. Proc. 18, 243, 1959). The enzyme contains vitamin B_{12} as a prosthetic group. The apoenzyme is present in extracts of mutant cells grown on methionine and has been purified 30-fold at pH 7.6 by ammonium sulfate fraction, adsorption on and elution from calcium phosphate gel and chromatography on hydroxylapatite and DEAE-cellulose. Purified apoenzyme was preincubated at 37°C. with vitamin B_{12} (10^{-6}M), a sulfhydryl compound (e.g. cysteine), tetrahydrofolate and FAD. Holoenzyme was apparently formed as measured by its activity when added to a methionine synthesizing system containing an antimetabolite of B_{12} which strongly inhibits further combination of free B_{12} with apoenzyme. All components of the preincubation mixture were necessary for optimal formation of holoenzyme. The DMBC coenzyme of Barker et al. (Proc. Nat. Acad. Sci. 45, 521, 1959) did not replace B_{12} for activation of methionine synthesis in a partially purified system.

6 *Biochemistry*

ABSORPTION AND TISSUE DISTRIBUTION OF RADIOACTIVE 5,6-DIMETHYLBENZIMIDAZOLE-B_{12} COENZYME. Kunio Okuda* and Bacon F. Chow. Johns Hopkins Univ., School of Hygiene, Balto., Md.

The absorption of cyanocobalamin (A) and 5,6-dimethylbenzimidazole-B_{12} coenzyme (B) as well as their tissue distribution was measured. a) In rats: norm. rats were fed by mouth 50 mmcg radiocobalt labeled A or B. Measurement of radioactivity in feces showed that less than 10% of B and approx. 30-50% of A were absorbed. When the same amt. of A or B was coadmin. with rat stomach ext. into the intest. loop (Fed. Proc. 18:588,1959), a similar difference in absorption was also observed. The comparison of distribution of radioactivity in various organs of rats sacrificed 6 days after a subcutaneous inj. of 500 mmcg of A or B demonstrated a greater hepatic uptake and a smaller renal uptake by animals receiving B than those receiving A, being 16.9±0.6% and 10.4±0.7% for hepatic uptake and 22.0±1.3% and 30.7±0.8% for renal uptake, resp. The fecal excretion of radioactivity in 6 days after the inj. was 7.9±1.0% for B and 13.6±0.4% for A. These data suggest that B has a greater affinity for the liver and that it is released via fecal route at a slower rate than A. b) In man: the urinary excretion test in healthy volunteers revealed that 1.5±0.2% of orally admin. dose of 5 mcg of B was excreted in 24 hour urine when 1 mg of A was used for flushing, and 1.9±0.2% when flushed with 1 mg of B.

7 *Biochemistry*

ENZYMES AND COFACTORS FOR METHIONINE BIOSYNTHESIS. F.T. Hatch, R.E. Cathou, A.R. Larrabee (intr. by L.G. Wesson), Div. of Biochemistry, Mass. Inst. of Technol. Cambridge 39, Massachusetts

Synthesis of the methyl group of methionine from the β-carbon of serine has been demonstrated in a soluble extract of E. coli strain 113-3 (which requires vitamin B_{12} or methionine for growth) cultured on vitamin B_{12} (Fed. Proc. 18, 243, 1959). Absolute requirements have been demonstrated for pyridoxal phosphate, tetrahydrofolate, DPNH, FAD, ATP, serine and homocysteine. Three enzyme fractions have been partially purified from the extract by means of ammonium sulfate fractionation, adsorption on and elution from calcium phosphate gel and chromatography on adsorbents: 1) serine hydroxymethylase, 2) an enzyme containing vitamin B_{12} as the prosthetic group and 3) a fraction which contains an enzyme lacking from a methionineless mutant of E. coli (strain 205-2 of B.D. Davis). The combined enzyme fractions catalyze methionine synthesis in the presence of the indicated substrates and cofactors. An additional enzymatic activity has been found which catalyzes the exchange of P^{32}-labeled pyrophosphate with ATP in the presence of homocysteine or methionine. The possible role of adenyl homocysteine and this newly recognized enzymatic component in the methionine synthesizing system is being investigated.

8 *Biochemistry*

UNBALANCED GROWTH IN LACTOBACILLUS LEICHMANII CULTIVATED IN EXCESS AND LIMITING VITAMIN B_{12} AND DEOXYRIBOSIDE. William S. Beck, Barbara Hurlock*, and Myron Levin*. Massachusetts General Hospital and Harvard Medical School, Boston, Massachusetts.

The rod-shaped organism Lactobacillus leichmanii (ATCC 7830) is commonly used in the turbidimetric microbiological assay of vitamin B_{12}, thymidine (TdR), and other deoxyribosides. In limiting B_{12} or deoxyriboside, elongated filamentous forms occur whose mean lengths are, within certain limits, inversely proportional to the B_{12} or deoxyriboside concentration. Study of this phenomenon shows that in a moderate excess of B_{12} (0.5 mμg. per ml.) or TdR (2.5 μg. per ml.), length averages 5.4 μ. In limiting B_{12} (0.005 mμg. per ml.) or TdR (0.05 μg. per ml.), lengths may increase to 150 to 200 μ. Further limitation produces smaller forms. In a massive excess of B_{12} (3.5 mμg. per ml.), sizes become unusually small (1 μ) with many approaching coccal form. These show increased viable counts per turbidity unit. B_{12} limitation decreases DNA synthesis, viability, and DNA/RNA ratios. Addition of excess B_{12} after growth in limiting B_{12} causes abrupt increases in DNA synthesis whose time-courses suggest synchronous growth. If excess B_{12} is added after the second hour, viability and normal size are not fully restored despite extensive DNA replation. The results show that average size is dependent upon B_{12} concentration and suggest that B_{12} functions in the biosynthetic pathway of DNA.

9 *Biochemistry*

ROLE OF VITAMIN B_{12} COENZYME AND BIOTIN IN PROPIONATE METABOLISM. E.R. Stadtman, P. Overath*, H. Eggerer* and F. Lynen.* Institut fur Zellchemie, Munchen.

Extracts of Propionibacteria catalyze an exchange of C^{14}-propionate into succinate (Phares, Delwiche and Carson). The exchange reaction in P. shermanii extracts is inhibited by avidin, and is lost after treatment with charcoal. It can be restored in the latter case by adding catalytic acetyl CoA and dimethylbenzimidazole-B_{12} coenzyme (DMBC). Since it is known that such extracts catalyze avidin-sensitive transcarboxylation of methylmalonyl CoA (MM CoA) to form propionyl CoA (Swick and Wood), and that liver MM CoA isomerase is impaired in B_{12} deficiency (Smith and Monty), the following sequence is proposed to account for the exchange: 1) Acetyl CoA+succinate $\rightleftharpoons$ succinyl CoA+acetate; 2) Succinyl CoA $\xrightleftharpoons{DMBC}$ MMCoA; 3) MM CoA+biotin-enzyme $\rightleftharpoons$ CO_2-biotin-enzyme+propionyl CoA; 4) Propionyl CoA + acetate $\rightleftharpoons$ propionate + acetyl CoA; sum: 5) Succinate + biotin-enzyme $\rightleftharpoons$ CO_2-biotin-enzyme + propionate. Reactions 1 and 4 are established. Evidence for (2) is that MM CoA forms succinyl CoA in presence of DMBC; for (3), that C^{14}-propionyl CoA lables MM CoA in absence of added DMBC; reaction (3) is avidin-sensitive.

XUM

10 *Pathology*

VITAMIN B_{12}-INTRINSIC FACTOR RELATIONSHIPS, Milton Toporek (intr. by J. W. Goddard). Dept. of Biochemistry, Jefferson Medical College, Philadelphia, Pa.

The isolated perfused rat liver technic (Miller, L.L., et al., J. Exptl. Med., 94, 431 (1951)) was used in these experiments. By itself in the perfusing blood, Co^{60}-B_{12} was taken up by the liver to only a very small extent, whereas in the presence of intrinsic factor (I.F. - soluble, purified hog I.F. from Lilly Research Labs.) the uptake of B_{12} was tremendously enhanced, confirming the work of others with liver slices and homogenates. The order of addition of B_{12} and I.F. to the perfusing blood is critical. B_{12} added first is apparently tied up by a factor in rat blood which renders it entirely unavailable to I.F. added 10 min. later, leading to very low uptake of B_{12} by the liver. The effectiveness of I.F. added first is only slowly destroyed, probably by the liver; B_{12} added 1 hr. after I.F. is absorbed to about 50% the usual extent if added together with I.F. After 2 hr. the effectiveness of I.F. is completely destroyed. Heating I.F. in Ringers (30 min. in boiling water bath), alone or with B_{12}, decreases the uptake of B_{12} by the liver only slightly, indicating I.F. to be quite stable to heat in this system. There is a rapid, significant transfer of radioactivity (up to 10% of dose in 2 hr.) from liver to bile. (Supported by U.S.P.H.S. Grant C-4236, and carried out with the technical assistance of Louis J. Philipp.)

11 *Nutrition*

EFFECT OF VITAMIN B_{12} AND ASCORBIC ACID ON THE UTILIZATION OF DIETARY PROTEIN IN 20 YOUNG WOMEN. Ellen H. Morse, Elna E. Daniels, Florence A. Hanlon, and Georgianna R. Walker (intr. by Helen S. Mitchell, U. Mass.) U. Conn., Storrs, Conn. (Contribution from Northeast Regional Project, NE-37.)

Two controlled diet experiments of 6 weeks each were conducted in the spring and fall of 1957. The daily protein intake was 40-45 gm, chiefly of vegetable protein to keep the dietary vitamin B_{12} at a minimum. Dietary ascorbic acid was 30 mg or less per day. Fat intake averaged about 30% of the total calories. After a standardization period of 2 weeks, the 20 college women were divided into 4 experimental groups and the diet was supplemented for 4 weeks as follows: Group I received 1000 γ vitamin B_{12}; II, 200 mg ascorbic acid; III, both supplements; IV served as controls. Responses to treatments were measured by nitrogen balance, red cell counts, hemoglobin, non-protein nitrogen and amino nitrogen in blood, and total serum cholesterol. The red cell counts showed a highly significant rise on vitamin B_{12} supplementation, but the effect of ascorbic acid alone or in combination was not significant. Serum cholesterol in all groups dropped significantly in two weeks and continued to have a slightly significant drop in the next 4 weeks. There was no significant difference in cholesterol, nitrogen balance, hemoglobin, non-protein nitrogen and amino nitrogen in blood among the 4 groups.

12 *Nutrition*

A RELATIONSHIP OF VITAMIN B_{12} TO HYDROXYMETHYL TETRAHYDROFOLIC DEHYDROGENASE. James S. Dinning and Rose Hogan*. Dept. of Biochemistry, Univ. of Arkansas School of Med., Little Rock, Ark.

Vitamin B_{12} has been shown to stimulate the conversion of formate to thymine methyl by bone marrow cells (Dinning and Young, J. Biol. Chem. 234, 1199) but to exert no stimulatory effect on the conversion of formaldehyde or serine beta carbon to thymine methyl (Dinning and Young, J. Biol. Chem. 234, 3241). These observations suggest that B_{12} functions in the reduction of one carbon compounds between the formate and formaldehyde levels of oxidation, a reaction mediated by hydroxymethyl tetrahydrofolic dehydrogenase (Hatefi et al., J. Biol. Chem. 227, 637). The activity of this enzyme, with anhydroleucovorin as substrate, has been found to be reduced in bone marrow and liver from B_{12} deficient chicks. The in vitro addition of B_{12} stimulated the enzyme in marrow but gave erratic results with partially purified enzyme from liver. The data suggest that B_{12}, or a derivative of the vitamin, may serve as a coenzyme for hydroxymethyl tetrahydrofolic dehydrogenase.

13 *Nutrition*

FURTHER STUDIES ON THE ABSORPTION OF INTRINSIC FACTOR (IF). O. Neal Miller, Frank J. Troncale*, and Hans J. Hansen*. Tulane Univ. Sch. of Med., New Orleans, La.

Vit. B_{12} injected through heart, portal vein, or femoral vein of the rat resulted in equal amounts of radio-B_{12} in the liver and the kidneys. B_{12}, bound to IF (hog), and injected into the femoral vein resulted in 11 x more vit. appearing in the liver and 9 x less appearing in the kidney. B_{12}, bound to lysozyme or condroitin sulfate, was without effect. Administration of B_{12}-IF complex via femoral vein causes somewhat less vit. B_{12} to appear in the liver than when it is administered by heart puncture. 95% of IF disappears from blood stream in 5 minutes. Attempts to show destruction of IF by liver have thus far failed. Liver, removed from an animal to which had been administered B_{12}-IF complex, was homogenized and fractionated by conventional techniques. 50% of the vit. was found in the microsomes, which is in contrast to the 2% that is observed when IF-B_{12} complex is incubated with liver homogenate in vitro. IF activity was found in the liver, following injection of IF into the portal vein of a rat, after the digestion of the microsome fraction with RNAse; 50 % of the injected activity was recovered. Microsomes, which had been isolated from liver of rats, 5 min. after injection of IF by portal vein, have been demonstrated to contain IF when tested by the Schilling technique on patients with P. anemia.

14 *Nutrition*

ON THE MECHANISM OF INTERACTION OF INTRINSIC FACTOR (IF) AND COFACTOR. Hans J. Hansen* and O. Neal Miller, Tulane Univ. Sch. of Med., New Orleans, La.

Hansen and Miller reported, (Fed. Proc., 18: 528, 1959) that the cofactor, which combines with IF appeared to be identical to ribonucleic acid (RNA). pH dependence of the combining reaction appeared to involve the PO_4 of RNA, suggesting the use of PO_4-gel as a model for study. Zirconyl phosphate-gel (Zgel), suspended in acetate buffer, was added to solutions of vitamin B_{12} (B_{12}), and IF. The amount of B_{12} combined to the Zgel was proportional to the amount of IF added. The activity of IF in this system was identical to that found in the RNA-assay and two other systems previously reported. RNA added at pH 7.0 competes with Zgel for IF-B_{12} complex. Competitive inhibition studies suggest that arginine, lysine and possibly histidine are apparently involved in combining IF-B_{12} to Zgel. Combining of B_{12}-IF to Zgel is maximal at pH 5.0 and decreases with increasing pH. The addition of 2 moles of Ca to one mole of zirconyl PO_4 results in a gel which will bind IF-B_{12} complex at pH 9.0 by a bond which is versene labile. A Ca bridge between the PO_4 of RNA (or DNA) and some group in IF such as SO_4 could explain the Ca dependent bond in liver slices and liver particulate assay systems.

1 258. VITAMINS–Vitamin C *Biochemistry*

CHARACTERIZATION OF AN ASCORBASE PRESENT IN THE FRUIT OF THE MALPIGHIA PUNICIFOLIA L. Conrado F. Asenjo, Arecio Peñaloza* and Dolores P. Medina*. Dept. of Biochemistry and Nutrition, School of Medicine, Univ. of Puerto Rico, San Juan 22.

The partial concentration and some of the properties of an ascorbase obtained from the fruit of the Malpighia punicifolia L. are described. The ascorbase activity is rapidly lost on storage of the fruit even when kept at a very low temperature. The ripe fruit has more ascorbase activity than the green one. The effect of substrate concentration, enzyme concentration, pH and temperature was studied in a Warburg respirometer using acetone powder preparations. The characteristics of this particular ascorbase resemble those of the ascorbic acid oxidases described in the literature although its activity is not inhibited by CN^+. (Supported by a grant from the Guggenheim Foundation)

259. VITAMINS–Vitamin D

1 *Physiology*

VITAMIN D AND THE TRANSFER OF Ca ACROSS THE INTESTINAL WALL IN VITRO. HAROLD E. HARRISON*, HELEN C. HARRISON AND EDWARD H. STEIN*, JOHNS HOPKINS UNIVERSITY SCHOOL OF MEDICINE AND BALTO. CITY HOSP., BALTIMORE MARYLAND.

EVERTED LOOPS OF SMALL INTESTINE FROM VITAMIN D DEFICIENT AND VITAMIN D TREATED RATS WERE FILLED WITH KREBS BICARBONATE BUFFER CONTAINING .02M GLUCOSE AND IMMERSED IN THE SAME SOLUTION. TRACER AMOUNTS OF CA^{45} WERE ADDED TO THE OUTER SOLUTION ONLY AND MOVEMENT OF CA^{45} TO THE INNER SOLUTION WAS MEASURED FOLLOWING INCUBATION WITH SHAKING IN 95%O2-5%CO2 ATMOSPHERE AT 37°C. THE RATE OF TRANSFER OF CA^{45} WAS MORE THAN TWICE AS RAPID IN INTESTINAL PREPARATIONS FROM THE VITAMIN D TREATED RATS. THE VITAMIN D EFFECT WAS DEMONSTRABLE 10 HOURS AFTER AN ORAL DOSE OF VITAMIN D BUT NOT AT 4 HOURS; IT WAS NOT BLOCKED BY CYANIDE NOR INCUBATION IN A 95% N_2-5% CO_2 ATMOSPHERE, NOR BY INCUBATION AT 10° OR 25°C. THE VITAMIN D EFFECT WAS FOUND IN LOOPS TAKEN FROM ALL PORTIONS OF THE SMALL INTESTINE, WHEREAS "ACTIVE TRANSPORT" OF Ca AGAINST A CONCENTRATION GRADIENT WAS FOUND ONLY IN THE PROXIMAL 10 TO 15 CM. OF INTESTINE. THE DATA SUGGEST THAT VITAMIN D INCREASES THE DIFFUSIBILITY OF Ca POSSIBLY BY A PERMEABILITY CHANGE WHICH IS NOT DEPENDENT ON OXIDATIVE METABOLISM.
(SUPPORTED BY A GRANT A-668 FROM THE N.I.H.)

2 *Biochemistry*

VITAMIN D AND THE STRUCTURE OF KIDNEY MITOCHONDRIA. Hector F. Deluca*, Sheldon Reiser* and Harry Steenbock. Dept. of Biochemistry, Univ. of Wisconsin, Madison.

In earlier experiments it was demonstrated that vitamin D either added *in vitro* or given to the rat reduced the oxidation of citrate by kidney homogenates and mitochondria. Further investigation revealed that this reduced oxidation did not appear to be due to an inhibition of an enzyme involved in the oxidation of citrate but rather due to a reduction of citrate penetration into the mitochondria. It has now been found by electron microscopic examination of thin sections of mitochondria isolated in 0.44 M sucrose that dietary vitamin D has a pronounced effect on the structure of kidney mitochondria. The vitamin D deficient mitochondria are swollen, have large spaces or vacuoles between the cristae and have a high frequency of disrupted internal structure. Vitamin D supplementation prevented these changes. It is suggested that citrate penetrates the vitamin D deficient preparations more readily thereby accounting for their elevated oxidation reported earlier.

260. VITAMINS–Vitamin E

1 *Biochemistry*

FUNCTION OF VITAMIN E IN MITOCHONDRIA AND MICROSOMES. A. L. Tappel, H. Zalkin*, and F. W. Knapp*. U. of Calif., Davis.

It is the purpose of this research to show how the large variety of enzymic and metabolic changes caused by vitamin E deficiency in experimental animals are related to the chemical properties of vitamin E. Vitamin E was found to inhibit unsaturated lipid peroxidation in mitochondria and the microsomal fraction and thereby stabilize their lipid-linked enzymes. Mitochondria and the microsomal fraction from the livers of vitamin E-deficient rabbits were found to be damaged by lipid peroxidation *in-vivo*. The mitochondria had decreased capacity for oxidative-phosphorylation with succinate. Mitochondria and microsomes held *in-vitro* deteriorated by cytochrome catalyzed lipid peroxidation. Free-radical intermediates of lipid peroxidation appeared to react at random with members of the electron transport chain. Studies of model systems show that the rates of unsaturated lipid peroxidation catalyzed by hematin compounds and the linoleate, hematin and tocopherol products which result are consistent with the hypothesis that this is the main pathological reaction of vitamin E deficiency (Arch. Biochem. Biophys. 47:223, 1953, 50:473, 1954). The mechanism by which dietary selenium compounds can replace vitamin E under certain conditions involves the formation of antioxygenic compounds *in-vivo*. Evidence for this mechanism was found with chicks on a vitamin E-deficient diet containing selenite.

2 *Biochemistry*

MALONALDEHYDE FORMATION AND THE INHIBITION OF GULONOLACTONE OXIDASE IN VITAMIN E-DEFICIENCY. R.E. Trucco, M.P. Carpenter*, P.B. McCay*, A.E. Kitabchi*, and R. Caputto. Okla. Med. Res. Inst., Oklahoma City, Okla.

Gulonolactone oxidase is inhibited in microsomes obtained from vitamin E-deficient animals. In these microsomes malonaldehyde production is increased, which indicates higher than normal lipid peroxidation. Tocopherol, EDTA, Co^{++} and Mn^{++} salts stop malonaldehyde formation and reactivate gulonolactone oxidase. Malonaldehyde was identified by the spectral and chromatographic behaviour of its combination with thiobarbituric acid and by its oxidation to malonic acid. Malonaldehyde is produced by interaction of the microsomal lipid with metals, or with ascorbic acid. In the presence of a thermolabile factor from microsomes, malonaldehyde is also produced by interaction of microsomal lipid and gulono- or glucuronolactones. By using a solubilized and partially purified gulonolactone oxidase preparation, the requirement of microsomal lipid for the inhibition has been ascertained. Malonaldehyde does not inhibit gulonolactone oxidase and, consequently, the role of direct inhibitor of this enzyme has been attributed to some intermediate in the formation of malonaldehyde. (Supported by grants from U.S.P.H.S. and Muscular Dystrophy Assns. of America.)

3 *Biochemistry*

THIOBARBITURIC ACID REACTION IN INCUBATED RAT LIVER HOMOGENATES. Laurence M. Corwin (Intr. by K. Schwarz). Natl. Insts. of Health, Bethesda, Md.

Production of the substance(s) giving the TBA reaction in incubated rat liver homogenates was studied. 10% homogenates were made up in 0.25 M sucrose, and 50 mg of tissue were incubated in a medium containing saline, Mg, and pH 7.4 phosphate buffer at 37.5°C for 1.5 hrs. Results are expressed as γ equivalents of malonaldehyde per 100 mg tissue. When vit. E-deficient homogenates were thus incubated, 4.1 γ were produced. The addition of succinate, DPN, and DPN plus α-ketoglutarate gave 4.2, 5.6, and 7.9 γ equiv. respectively. Dietary vit. E reduced α-KG and succinate supplemented homogenates to 0.3 and 0.1 γ respectively. Dietary selenite had no effect. *In vitro* addition of 5 γ tocopherol or 10 γ of the tocopherol metabolite isolated by Simon et al., with α-KG as substrate, reduced the color reaction to half. DPPD (0.5-1 γ) lowered it to 0.4 γ. Mitochondria, microsomes and supernatant, when incubated separately, formed 0.7, 0.9 and 1.5 γ. Mixing of mitochondria with microsomes or supernatant gave 2.5 and 4.4 γ. All three combined gave 9.1 γ. Factors such as citrate or buffered .25 M sucrose, which inhibit swelling of mitochondria, also diminished substances reacting with TBA. There was lack of correlation between the TBA reaction and the decline of succinate and α-KG oxidation seen in E-deficient homogenates.

4 *Nutrition*

MUSCULAR DYSTROPHY IN CHICKS INFLUENCED BY ARGININE. C. C. Calvert*, M. C. Nesheim* and M. L. Scott, Cornell University, Ithaca, New York.

It was shown in chicks fed a purified diet containing casein as the principle source of protein that when the diet was deficient in arginine, sulfur amino acids and vitamin E no muscular dystrophy occurred up to 7 wks. of age. When this diet was supplemented with 1, 2, or 4 percent arginine monohydrochloride, severe muscular dystrophy occurred in 90% of the chicks by the time they were 5 wks. of age. The levels of 2% and 4% arginine did not increase the incidence or severity of muscular dystrophy over that obtained with 1% arginine. When chicks were fed the basal arginine-deficient diet to 5 wks. of age and then received the basal diet supplemented with 1% arginine, 7 out of 10 chicks receiving the arginine supplement showed muscular dystrophy within the next 2 wks. (by the time they were 7 wks. of age), whereas none of the chicks receiving the arginine-deficient diet developed symptoms of dystrophy. When the basal diet containing arginine was supplemented with either methionine, cystine or vitamin E, muscular dystrophy was prevented. These results, therefore, indicate the existence of a very important interrelationship between the sulfur amino acids, arginine and vitamin E in muscular dystrophy. (Supported by grants from the Muscular Dystrophy Association of America and the Nutrition Foundation, Inc.)

XUM

5 *Nutrition*

EFFECT OF COCCIDIOSIS INFECTION ON VITAMIN A AND E LEVELS OF THE RABBIT: J. Friedrich Diehl and Bill G. Kistler (Intr. by J. S. Dinning), Dept. of Biochemistry, University of Arkansas School of Medicine, Little Rock, Ark.

In continuation of earlier experiments (Fed. Proc. 18, 523 (1959)] we have studied the effect of hepatic coccidiosis on vitamin A and E levels in young rabbits. Severe infections with Eimeria stiedae led to extremely low vitamin E levels in liver and serum and to low vitamin A levels in liver of rabbits kept on regular laboratory chow. Variable degrees of lowering of vitamin levels were found in less severe infections. Saturation tests with oral and parenteral administration of vitamin E indicated that the hypovitaminosis E of coccidiosis-infected rabbits was not merely due to malabsorption. Liver damage or a direct effect of the parasite may be contributing factors. The hypovitaminosis A may be a secondary phenomenon, as vitamin E normally exerts a protective effect on vitamin A.

6 *Nutrition*

SOME HISTOLOGICAL CHANGES IN THE VITAMIN E DEFICIENT MONKEY. Coy D. Fitch,* James S. Dinning, Ruth S. Young,* Paul L. Day, and Horace N. Marvin.* Depts. Biochemistry and Anatomy, Univ. of Arkansas, School of Med., Little Rock, Ark.

Young rhesus monkeys fed a purified diet deficient in vitamin E, but with a relatively high fat content, develop a syndrome characterized by marked weakness, skeletal muscle wasting, creatinuria, and anemia. Histological sections from the various tissues of these animals have been compared to tissues of similar monkeys receiving the same diet supplemented with vitamin E, one monkey which had been recovered from vitamin E deficiency, and one monkey fed a regular diet. The most striking changes were in skeletal muscle and bone marrow. Skeletal muscle from the deficient animals showed increased basophilia, loss of cross striations, and areas of necrosis. There was an increase in sarcolemma nuclei with aggregations of 10-15 nuclei in some instances. No such lesions were seen in cardiac muscle. The bone marrow showed increased cellularity with a disproportionate increase in cells of the granulocytic series.

7 *Nutrition*

VITAMIN E AND ANTIOXIDANTS IN THE NUTRITION OF THE RAT. O. E. Crider*, Petar Alaupovic*, and B. Connor Johnson. Dept. of Animal Science, University of Illinois, Urbana.

It has been found that rats carried through resorption-gestation on vitamin E-deficient diets will respond in a second reproduction cycle to the antioxidants N,N'-diphenyl-p-phenylenediamine and 1,2-dihydro-6-ethoxy-2,2,4-trimethylquinoline, but not to butylated hydroxytoluene or acid-reduced ubiquinone (chromane structure), by bearing live young. In addition, rats have been carried through three generations, following resorption-gestation in the original dams, on vitamin E-free diets containing DPPD. The livers and carcasses of these rats, of control rats on checkers, of vitamin E-deficient rats, and of rats receiving high levels of α-tocopherol were all found on assay to contain α,α'-dipyridyl-reacting material. Chromatographically, however, essentially none of this α,α'-dipyridyl-reacting material was vitamin E, except in the rats which had received high levels of α-tocopherol. In the case of the DPPD-fed animals DPPD was found chromatographically. Thus it has been found possible to separate and detect at least three α,α'-dipyridyl-reacting materials, α-tocopherol, DPPD and an unidentified compound in rat livers and carcasses.

8 *Nutrition*

DETERMINATION OF N,N'-DIPHENYL-p-PHENYLENEDIAMINE (DPPD) IN ANIMAL TISSUES AND ITS BIOPOTENCY IN MUSCULAR DYSTROPHY OF RABBITS. H. H. Draper and A. Saari Csallany.* Division of Animal Nutrition, University of Illinois, Urbana.

A sensitive method has been developed for determination of the vitamin E-active antioxidant DPPD in rat liver, based on spectrophotometric measurement of the color developed by oxidation with ferric chloride in ethanolic solution. The subcellular distribution of orally administered DPPD was found to be similar to that of total lipid, i.e., over half was located in the supernatant, about one-quarter in the microsomal fraction and a small portion in the mitochondria. Administration of DPPD to vitamin E-deficient dystrophic rabbits fed a basal diet free of tocopherols led to remission of gross symptoms in 5 days, of hypercholesterolemia in 3 weeks and of susceptibility of red blood cells to hemolysis by dilute hydrogen peroxide within 2 weeks. The results confirm previous findings indicating that the vitamin E requirement of rabbits and rats represents a need for suitable antioxygenic compounds. (Supported by a grant from the Muscular Dystrophy Associations of America, Inc.).

9 *Nutrition*

ENZYME STUDIES OF SKELETAL MUSCLE IN VITAMIN E DEFICIENCY T. R. Koszalka*, G. Krol*, AND K. E. Mason. Div. of Experimental Radiology, and Depts. of Biochemistry and Anatomy, Univ. of Rochester School of Medicine and Dentistry, Rochester, N.Y.

Proteolytic and autolytic activity was studied in skeletal muscle of vitamin E-deficient hamsters and rats. Proteolytic activity was determined at pH 3.5-3.8, using hemoglobin as substrate. Autolysis was studied at pH 9.0 without addition of substrate. Muscle proteolytic activity of hamsters on an E-deficient diet for 50-60 days was similar to that of controls on stock diet or controls on the experimental diet supplemented with vitamin E. However, a two-fold increase was observed after 120-130 days, and a ten-fold increase after 327-354 days, of vitamin E deficiency. In hamsters deficient for 330 days, vitamin E therapy for 10-40 days produced a decrease of approximately 72% in proteolytic activity. Therapy with alpha-tocopherylhydroquinone has a similar effect. Autolytic activity of muscle varied in the same direction as the proteolytic activity, but not to as striking a degree. Similar results were obtained with rats. (Supported by a grant from the Muscular Dystrophy Associations of America, Inc.)

10 *Nutrition*

PATHOLOGY OF VITAMIN E DEFICIENCY IN THE MINK. H. D. Stowe,* C. K. Whitehair and H. Travis.* Michigan State Univ., East Lansing, Mich.

Experiments were conducted using 4-week-old mink to determine vitamin E deficiency symptoms and lesions. They were fed semipurified vitamin E deficient rations with casein, Drachett protein and Torula yeast as sources of protein. Acute deaths were encountered in mink fed the basal vitamin E deficient ration containing Drachett protein and Torula yeast. Gross tissue lesions were mainly degenerative changes of the myocardium and some skeletal muscles. Micropathology of these tissues showed hyaline and vacuolar degeneration, focal calcification, loss of striation and swelling of the muscle fibers and acidophilic sarcoplasm. Weight gains were not affected by the alpha tocopheral deficient rations. Blood alpha tocopherol values were correlated directly with amounts supplemented in the ration. Supplementing 1 PPM selenum to the basal ration minimized vitamin E deficiency symptoms. Supplementing 4% unsaturated fat to the basal ration produced a different syndrome characterized by nonsuppurative inflammation of the subcutaneous and visceral fat, edema and pigmentation of the affected adipose tissue. The alpha tocopherol requirements for mink appear to be less than 2.5 mg. alpha tocopherol per 100 gm. ration. (Supported by a grant from the Mink Farmers Research Foundation.)

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Nutrition

11

A TOCOPHEROL SPARING EFFECT OF S-AMINO ACIDS. K. Schwarz and C. M. Foltz*. Natl. Insts. of Health, Bethesda, Md.

In rats on a vit. E and Factor 3/selenium deficient Torula yeast diet, prevention of liver necrosis by L-cystine is largely due to contamination of the cystine with Se (K. Schwarz et al., Metabolism 8, 88, 1959). However, Se-free supplements of .5% L-cystine or .62% D,L-methionine, while not preventing death, increased survival from 22 to 33 days, which indicated a sparing effect on vit. E or Factor 3. Comparison of diets with graded dose levels of α-tocopheryl acetate, with and without methionine, proved that the S-amino acid reduces the vit. E requirement to ca. 1/10th of that normally observed. While .75 mg are required per 100 g of diet to afford 50% protection without added S-amino acid, only 72 μg exert this effect in the presence of .62% D,L-methionine, and 300 μg% vit. E protect over extended periods of time. For the vit. E potentiating effect, the S-amino acids are needed in excess of the requirement for growth. There was no difference in results with Se-free methionine, homocystine, cystine and cysteine, added to a diet with 150 μg% vit. E. Taurine, cystamine, sulfite, sulfate, and homoserine, serine and choline were inactive. Parallel experiments with small levels of selenite or other sources of Factor 3-activity gave only minor effects.

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EFFECT OF LONG CHAIN KETO ACIDS ON THE SEVERITY OF CHICK ENCEPHALOMALACIA. M. Kokatnur*, M. Uchiyama*, S. Okui*, H. Tsuchiyama*, F. A. Kummerow, and H. M. Scott. Depts. of Food Technology and Animal Science, University of Illinois, Urbana, Ill.

Groups of week-old male chicks were kept on a diet which consisted of 30% protein (isolated soybean protein), 10% corn oil, 55% glucose (cerelose) and all of the essential minerals and nutrients except for inositol, vitamin E and vitamin C. In one series, this diet was supplemented with 0.25% of various long chain keto acids and in another the most potent keto acid was fed at three different levels of corn oil. The results indicated that 0.25% of 12-oxo-cis-9-octadecenoic acid or its methyl ester was the most effective keto acid in inducing encephalomalacia. All chicks which received this keto acid showed positive symptoms of encephalomalacia within one week. The dietary level of corn oil seemed to be an important factor in the development of symptoms as encephalomalacia developed more quickly at a 10% than at a 2.5% level of corn oil. (Supported by grant H-1819, National Institutes of Health, Dept. of Health, Education, and Welfare.)

Nutrition

13

THE INTERRELATIONSHIP OF VITAMIN E, LINOLEIC AND LONG CHAIN KETO ACIDS. M. Sugai*, M. Inoue*, H. Tsuchiyama*, and F. A. Kummerow. Dept. of Food Technology, University of Illinois, Urbana, Ill.

Both chicks and rats were used in these studies. Eight groups of week-old chicks were kept on a diet which consisted of 20% protein, 70% glucose, 1% lard stripped of vitamin E and all of the essential minerals and nutrients except vitamin E. The diets of 4 of the 8 groups of chicks were supplemented with 1.5% methyl linoleate and the other 4 with 0.25% 12-Oxo-cis-9-octadecenoic (keto) acid. In addition three lots in each group of chicks were supplemented with α-tocopherol acetate, cholesterol, or both. Weanling as well as mature rats were fed an adequate diet and various levels of corn oil and keto acid. The results indicated that the presence of a dietary source of vitamin E but not cholesterol influenced the physiological response to linoleic acid. Approximately 80% of the chicks which had received methyl linoleate or 0.25% keto acid developed symptoms of encephalomalacia and died within six weeks. Although α-tocopherol was able to protect chicks against these symptoms in the presence of methyl linoleate, it provided no protection in the presence of the keto acid. This keto acid caused intestinal hemorrhage in both weanling and adult rats. (Supported by NIH grant H-3063.)

Biochemistry

1

THE ROLE OF VITAMIN A IN NUTRITIONAL HYPOPROTHROMBINEMIA IN RATS. John T. Matschiner* and E. A. Doisy, Jr. Dept. of Biochemistry, St. Louis Univ. Sch. of Med., St. Louis, Mo.

Difficulties in the development of symptoms of vitamin K deficiency in rats have led to a study of the effects of vitamin A in such experiments. Toxic doses of vitamin A are known to induce hemorrhage in rats and the condition is prevented by the administration of vitamin K. Weanling male rats from our colony were fed a purified diet deficient in vitamin K and containing vitamin A either as the crystalline acetate or as a concentrate (Upjohn, Super A). Prothrombin in plasma was measured by the method of Hjort et al. (J. Lab. Clin. Med., 46, 89 (1955)). Marked hypoprothrombinemia was present after eight weeks in animals which received approximately 500 I.U. per day but not in animals receiving no vitamin A or 5 I.U. per day. Severe hypoprothrombinemia occurred at 5000 I.U. per day and 60% hemorrhagic mortality occurred at the 15,000 I.U. level. This latter amount is within the range of levels previously used to induce hemorrhage in the rat. The effect of vitamin A was greater with the more hemorrhagenic diet of Johnson, et al. which contains beef preserved by irradiation (J. Nutrition 69, 18 (1959)).

Nutrition

2

THE CAUSE OF VITAMIN K DEFICIENCY IN MALE RATS FED IRRADIATED BEEF. M. S. Mameesh*, V. C. Metta*, P. B. Rama Rao*, and B. Connor Johnson. Dept. of Animal Science, Univ. of Illinois, Urbana.

It has been established that male rats fed a synthetic diet containing 35% beef irradiated at 2.8 or 5.6 megarad with no added vitamin K die of hemorrhages and that this syndrome is preventable and curable by vitamin K (Fed. Proc. 18, 537(1959). This syndrome has been produced in rats fed purified, K-free diets in the absence of beef or any irradiated material, thus demonstrating a dietary vitamin K requirement by the rat. Beef irradiated with either 2.8 or 5.6 megarad was found to contain half as much vitamin K activity as control beef according to chick assays. Coprophagy, an important source of vitamin K for the rat, was found to be reduced in rats fed diets containing 5.6-megarad-irradiated beef when compared to diets containing 2.8-megarad-irradiated or control beef. The vitamin K requirement of the rat was quantitatively determined on a K-free diet and was found not to be increased by feeding irradiated beef. From all these considerations it appears that the hemorrhagic syndrome which develops in rats fed irradiated beef is due partially to vitamin K destruction by irradiation and partially to the failure of the rat to consume his feces on diets containing highly irradiated beef.

Nutrition

3

EFFECTS OF HORMONES AND STRAIN OF RAT ON THE INCIDENCE OF THE HEMORRHAGIC SYNDROME IN MALE RATS FED IRRADIATED BEEF. Om P. Malhotra* and Elwood F. Reber. College of Veterinary Medicine, University of Illinois, Urbana, Illinois.

Thirty Holtzman (H) and 32 Sprague-Dawley (SD) weanling male rats were fed a ration containing 35% irradiated (5.58 megarads) beef for 8 weeks. 20% of the H and 56% of the SD rats died due to hemorrhages. The prothrombin time (PT) was prolonged in 4% of the H and 23% of the SD rats. 64 H and 58 SD rats were used to evaluate the effects of castration and the administration of stilbesterol or testosterone on the incidence of hemorrhages. The following percentages of rats died in the designated treatments: intact + testosterone, 88 SD and 27 H; castrated + testosterone, 70 SD and 28 H; intact, 62 SD and 7 H; castrated, 37 SD. No rats died in the following treatments: castrated, H; intact + stilbesterol; castrated + stilbesterol; or controls. In general the incidence of prolonged PT followed the same pattern as the occurence of deaths. The stilbesterol treated rats gained an average of 16 gms for the 5 weeks period. The remainder of the rats gained 130-200 gms for the same period. The effect of testosterone in producing hemorrhages was independent of the weight gain. The SD strain was more susceptible to the hemorrhagic disease than was the H strain. The incidence of the disease was increased by testosterone.

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The list of categories by which the abstracts are arranged is given as the table of contents at the beginning of the Abstracts section.

ABOUT THIS INDEX

Readers may be interested to know of some unique features about the preparation of this index. It is an experiment in several of its aspects. The authors of this year's abstracts had a major part in determining the subject categories into which their abstracts are placed, and, also, the subject headings under which their abstracts are listed in the index. A Remington Rand UNIVAC Electronic Computer was used to arrange the data for this volume, i.e. assign abstract numbers, page numbers, compile the index, etc. The editors are aware of many small imperfections in the final product, but feel that as a first attempt to use new techniques, the experiment has been highly successful. This subject index is more detailed and more carefully cross-referenced than any previous index to FEDERATION PROCEEDINGS. If you are inclined to give us your reaction to it, the Editorial Office would be pleased to hear from you.

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XUM

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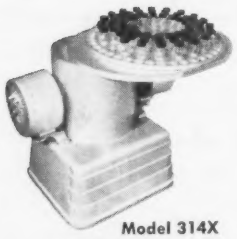

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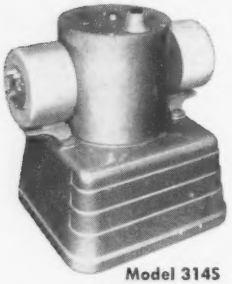

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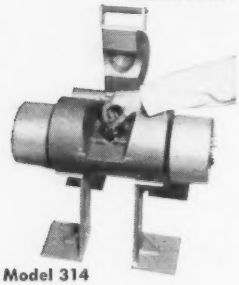

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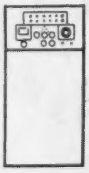

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